ENRICHMENT OF LIVE FEEDS WITH VARIOUS OIL EMULSIONS: EFFECTS ON YELLOWTAIL FLOUNDER LARVAE, AND ON ROTIFERS AND BRINE SHRIMP

METUSALACH

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By

©METUSALACH, B.Sc. (Hon.), M.Sc.

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Department of Biology
Memorial University of Newfoundland
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St. John's Newfoundland Canada

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DEPARTMENT OF BIOLOGY

MEMORIAL UNIVERSITY OF NEWFOUNDLAND

ST. JOHN'S, NEWFOUNDLAND, CANADA

APRIL 2002

DEDICATED TO MY LOVING:

WIFE, DAHNIAR NUR

SON, ILHAM AKBAR MINANGA

& DAUGHTER, NURUL FATIHA MINANGA

Abstract

The production of live feed for use in the culture of marine finfish larvae is critical. Live feed needs to be of high quality to promote good growth, survival and pigmentation. The effect of live feed enrichment on yellowtail flounder (*Limanda ferruginea*) larvae was studied. Live feeds were enriched with seal oil, menhaden oil and Algamac (algal-based formulated diet) in one experiment, and with seal oil, seal oil+DHASCO (docosahexaenoic acid-rich single cell oil) and Algamac in a second experiment. The survival of larvae ranged from 5.7 to 20%. The specific growth rate (SGR) of larvae fed Algamac was higher than that of seal oil-fed larvae in the first experiment, but was similar with other treatments in the second experiment. The proportion of fish having complete pigmentation and eye migration was 43 – 53% and 56 – 72%, respectively. The n-3 highly unsaturated fatty acid (HUFA), essential fatty acid (EFA) contents and EFA ratios in rotifers correlated with complete eye migration of fish in the first experiment, whereas only arachidonic acid (AA) content was found to correlate with normal pigmentation in the second experiment flounder.

The fatty acid composition of the total lipid of larvae in each feeding reflected, in general, the fatty acid profile of their respective diets. The EFA contents of larvae fed seal and menhaden oil were similar ($p>0.05$), whereas those of larvae fed Algamac were higher ($p<0.05$). The fatty acid composition of normally and abnormally pigmented flounder fed the same diet was generally similar. In all feeding regimes, 16:0, 18:1n-9 and 18:3n-3 were the most abundant fatty acids present.

Enriched rotifers varied in their total lipid contents among the enrichment concentration and period. While the effect of enrichment concentration was absent in the DHASCO treatment ($p=0.15$), the effect of enrichment period was observed in all

treatments ($p \leq 0.01$). The EFA contents in rotifers were influenced by both the enrichment concentration and period ($p < 0.03$). An interaction between the enrichment concentration and period exerted a significant effect on the EFA contents ($p \leq 0.03$) of rotifers, except on the DHA of the Algamac-fed rotifers ($p = 0.11$).

The total lipid contents of *Artemia* generally affected ($p < 0.001$) by both the enrichment concentration and period. Fatty acids 16:0, 18:1n-9 and 18:3n-3 were the primary constituents of *Artemia* lipids. The content of EFA in *Artemia* fed with different oil emulsions was affected ($p < 0.001$) by both emulsion concentration and enrichment period. A significant interaction existed between enrichment concentration and period on the total lipid and EFA contents ($p < 0.01$), except on the EPA of the seal oil-fed *Artemia* ($p = 0.06$).

This study demonstrates that using seal oil and menhaden oil to enrich live feeds gives comparable results on the general performance of yellowtail flounder larvae to those when using commercial enrichments containing high DHA levels, and that this species does not require high dietary DHA for maximal survival, growth and pigmentation. Results also suggest that the concentration of enrichment medium and length of feeding period affect the total lipid, DHA, EPA and AA contents in live feeds.

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List of Abbreviations

AA	Arachidonic acid (20:4n-6)
AM	Algamac
AP	Abnormally pigmented
CEM	Complete eye migration
CP	Complete pigmentation
CS_2	Carbon disulphite
DHA	Docosahexaenoic acid (22:6n-3)
DHASCO	DHA-rich single cell oil
DPA	Docosapentaenoic acid (22:5n-3)
DPFF	Day post first feeding
DW	Dry weight
EFA	Essential fatty acid
EPA	Eicosapentaenoic acid (20:5n-3)
FA	Fatty acid
GC	Gas chromatography
HUFA	Highly unsaturated fatty acid
MO	Menhaden oil
MUFA	Monounsaturated fatty acid
NP	Normally pigmented
PFF	Post first feeding
PUFA	Polyunsaturated fatty acid
SD	Standard deviation
SFA	Saturated fatty acid
SGR	Specific growth rate
SO	Seal oil
TBHQ	Tert-butylhydroquinone
wt.	Weight

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Chapter 1

General Introduction

1.1 Background

The depletion of fish stock as well as continuing strong consumer demand for high value fish have fostered aquaculture efforts world-wide. The marked development of fish culture is causing an increasing demand for good quality fish fry. Fish fry production depends largely on the success of larval rearing, which is greatly influenced by first feeding regimes and the nutritional quality of starter diets (Izquierdo *et al.*, 2000); dietary lipids being recognized as one of the most important nutritional factors that affect larval growth and survival (Watanabe, 1991; Izquierdo, 1996; Sargent *et al.*, 1997, 1999a; Bell, 1998). The success of aquaculture ventures requires the optimization of growth and health of fish at all stages of their life history (Waters, 1996). Substantial progress has been made in a number of areas, such as larval nutrition and the control of spawning (Dhert *et al.*, 1994; Kanazawa, 1995; Rainuzzo *et al.*, 1997; Sargent *et al.*, 1997; Gara *et al.*, 1998; Mangor *et al.*, 1998; Masuda and Tsukamoto, 1998; Rønnestad, *et al.*, 1998a).

The main obstacle in the mass production of juveniles of marine fish for commercial culture has been the low survival of larvae during the yolk-sac and first feeding stages. Marine fish larvae are, in general, small, vulnerable and susceptible to environmental stress (Bell, 1998). High larval mortality during the first feeding period is due, in part, to the lack of a nutritionally adequate live food organism. Many studies have focused on nutritional requirements of larvae during the first feeding period in order to

uncover the best possible feeding regimes to ensure good survival and growth. These fish larvae require food organisms that have relatively high concentrations of essential fatty acids (EFAs) such as eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid (AA) (Sargent *et al.*, 1995a,b, 1997, 1999a,b; Izquierdo, 1996; Izquierdo *et al.*, 2000). Good survival during larval stages and fast growth are among the characteristics for a species to be an ideal candidate for aquaculture.

1.1.1 Live feeds

The nutritional quality of cultured live prey for rearing larval fish depends on the transfer of dietary components from their food to their body (White and Nagata, 1990). Although proximate chemical composition of live prey and their diet may not be similar, constituent units of dietary macronutrients, particularly fatty acids of lipids, are known to be transferred (Watanabe *et al.*, 1983). Despite efforts to develop artificial alternatives (Léger *et al.*, 1986; Jones *et al.*, 1993), rotifers and *Artemia* nauplii remain important in aquaculture as a live food for fish larvae. However, a major drawback in the use of *Artemia* nauplii is variability in their highly unsaturated fatty acid (HUFA, with 4 or more double bonds) content (Léger *et al.*, 1986; Webster and Lovell, 1990), and a number of studies have shown that it is the HUFA content of *Artemia* nauplii that determines their food value to fish larvae. *Artemia* nauplii do not provide adequate nutrition for fish larvae, and there is now good evidence to show that this results from deficiency in EFA.

The nutritional value of diets of larvae and fry may be enhanced by enrichment with n-3 HUFA (Narciso *et al.*, 1999). Live feeds such as *Artemia* and rotifers are able to

absorb these important dietary constituents from an aqueous emulsion to which n-3 HUFA have been added. The fatty acid composition of live feeds is extremely important because n-3 PUFAs, particularly EPA (20:5n-3) and DHA (22:6n-3), are essential for early larval growth of many fish species (Ben-Amotz *et al.*, 1987; Watanabe, 1991). Live feeds such as rotifers are able to synthesize n-3 PUFA, but tissue levels of n-e HUFA and EFA are insufficient to meet the demands for normal growth and development of fish larvae (Lubzens *et al.,* 1985). Both rotifers and *Artemia* are known to have little ability to elongate and desaturate fatty acids, but they can adopt the fatty acid profile of their diets (Léger *et al.,* 1986; Lubzens, 1987). Tissue accumulation of these acids by live feeds is therefore dependent on exogenous supplies. In addition to energy and essential fatty acids, rotifers and *Artemia* transfer vitamins and minerals to fish larvae if these compounds are supplied in the emulsions fed to the live prey.

1.1.2 Dietary lipids

Lipids are among the most important components of fish larvae during their development. Fish, like other animals, use lipids for energy, cellular structure and maintenance of the integrity of cell membranes. While neutral lipids such as triacylglycerol and free fatty acids are important energy reserves, phospholipids, in particular, are very important since they are involved in membrane formation, provision of energy and synthesis of biologically active hormone-like substances, such as prostaglandins, thromboxanes and leukotrienes, collectively known as eicosanoids. Membrane fluidity is, in part, regulated by the fatty acid composition of the

phospholipids, which control such processes as cellular transport and the activities of membrane-associated enzymes. The degree of fatty acid unsaturation in fish tissue increases when environmental temperature decreases, thereby maintaining membrane fluidity to allow normal cellular function (Næss and Lie, 1998).

Due to the importance of dietary lipid utilization for larval rearing success, increased attention has been paid to different aspects of larval lipid nutrition such as digestion, absorption, transport and metabolism of dietary lipid. The capacity for lipid absorption by the intestinal epithelium has been observed at the onset of exogenous feeding, and the capacity to absorb lipid increases with development in larvae. However, this increase in capacity is delayed in formulated diet-fed larvae (Izquierdo *et al.*, 2000).

Dietary lipid utilization by larvae may be directly or indirectly affected by several morphological and physiological changes that occur during larval development. For instance, throughout larval development, the number of intestinal folds is increased, the stomach is formed and its function improved accordingly, and these changes in enterocytes and the digestive system imply an improvement in the digestion and absorption efficiency of the juvenile (Izquierdo *et al.*, 2000). As a consequence of these changes, variations may be expected in the efficiency of digestion, absorption, transport or metabolic utilization of dietary lipid during larval development. Many studies regarding the critical role of lipids, in particular that of PUFA, during the development of larval fish have appeared (Bell *et al.*, 1986; Watanabe, 1993; Sargent, 1995; Izquierdo, 1996; Bell, 1998). Sargent (1995) has briefly summarized the PUFA requirements of fish as follows:

a. Fish have an absolute requirement for n-3 PUFA and almost certainly for n-6 PUFA.
b. Freshwater fish are generally capable of converting 18:3n-3 and 18:2n-6 acids to their higher homologues 22:6n-3 and 20:4n-6, respectively.
c. Those marine fish so far studied are incapable of these conversions and require a dietary source of the end products 22:6n-3 and 20:4n-6 (arachidonic acid, AA).

Fish, like all other vertebrate studied so far, require three long chain PUFA, namely DHA, EPA and AA, for their normal growth, development and reproduction (Sargent *et al.*, 1993a,b, 1995a,b, 1997). In fish, as in terrestrial mammals, DHA, EPA and AA are all involved in maintaining cell membrane structure and function. However, DHA and EPA and not AA are the major PUFA of cell membrane in fish. The converse is true in terrestrial mammals except in their neural tissue including the eye where DHA can be very abundant (Sargent *et al.*, 1999a).

In animals, including fish, PUFA of the n-3 and n-6 series cannot be synthesized *de novo* and must, therefore, be obtained from the diet and are regarded as essential fatty acids (EFA). These EFA are an absolute requirement to maintain normal cellular structure and function. In freshwater fish, the EFA requirements can be met by supplying α-linolenic (18:3n-3) and linoleic (18:2n-6) acids at a concentrations of around 1-2% in the diet by weight (Sargent *et al.*, 1995a; Bell, 1998). The EFA requirements of marine fish, in contrast, can only be met by providing the long chain HUFA, DHA and EPA, at concentrations of 0.5-1.7% in the diet (Sargent *et al.*, 1995a; Bell, 1998). The requirement for these HUFA arises from the fact that in all marine fish studied to date, there is only very low Δ5-desaturase and/or C:18 to C:20 elongase activities, which are

required for the production of EPA (Tocher *et al.*, 1989; Sargent *et al.*, 1995a, 1997). There is considerable evidence, however, that while the conversion of EPA to DHA is possible in marine fish, the conversion rate may be too low to meet the high demand for the latter, particularly in rapidly growing and developing larvae and juveniles (Sargent *et al.*, 1993b, 1997). The abundance of 20:5n-3 and 22:6n-3 in the marine food web presumably negates the requirement for a high Δ5-desaturase or C:18 to C:20 elongase activity in marine fish (Sargent *et al.*, 1995b).

The essentiality of dietary n-3 HUFA for marine fish has been reviewed by many authors (e.g., Rimmer and Reed, 1990; Webster and Lovell, 1990; Lemm and Lemarie, 1991; Watanabe and Kiron, 1994; Izquierdo, 1996, Sargent *et al.*, 1999a,b). Marine fish eggs are characterized by their high contents of EPA and DHA, which are retained throughout embryonic development, particularly DHA, suggesting the importance of these fatty acids for the developing embryo (Rainuzzo *et al.*, 1993). The AA, EPA and, particularly, DHA are also preferentially retained in the larval lipids at the expense of other fatty acids during starvation periods (Tandler *et al.*, 1989), allowing the preservation of essential components of biological membranes during such critical periods. Deficiency in n-3 HUFA retards fish growth, reduces resistance to stress and induces mortality (Izquierdo, 1996). In addition, HUFA deficiency has been associated with some anatomical alterations such as hypomelanosis on the ocular side of flatfish (Kanazawa, 1993b). DHA is known to have a higher efficiency than EPA as an essential fatty acid (Watanabe *et al.*, 1989; Watanabe, 1993), the former being particularly

accumulated in the oldfactory nerve, retina and central nervous system (Sargent *et al.*, 1993a).

Besides a minimum dietary requirement for each essential fatty acid, the relative proportions among the different PUFA in larval tissues seems to be related to the best growth rates. The competitive interaction among fatty acids, especially EFA (Iijima *et al.*, 1998), necessitates control of both their dietary proportions as well as absolute amounts.

1.1.3 Pigmentation

One of the most common defects in hatchery-reared fish is malpigmentation, particularly albinism (hypomelanosis). Malpigmentation of cultured flatfish juveniles continues to be a major problem for mariculturists due to its irreversible nature and undesirable appearance (Næss and Lie, 1998). It also reduces the market value of affected fish (Seikai, 1991; Seikai and Matsumoto, 1991; Næss *et al.,* 1995; Næss and Lie, 1998). In the wild, albino juveniles are easily seen by predators. This contributes to poor survival rates when hatchery-raised fish are used to supplement wild stocks or enhance coastal fisheries (Seikai *et al.,* 1987a; Seikai and Matsumoto, 1991; Howell, 1994; Furuta, 1998; Furuta *et al.,* 1998).

Larval nutrition has been implicated as a possible factor in determining normal pigmentation patterns, since flatfish larvae fed a diet of wild zooplankton generally exhibit a higher rate of normal pigmentation compared to larvae fed *Artemia* nauplii (e.g., Seikai *et al.,* 1987b with Japanese flounder; Næss *et al.,* 1995 with Atlantic halibut).

Pigmentation is thought to be under both neural and hormonal control (Estevez *et al.*, 1997) and thus may be influenced by dietary essential fatty acids levels. Reitan *et al.* (1994) obtained a positive correlation between DHA/EPA ratios in turbot larvae and their subsequent pigmentation rates.

The composition of diets for larval rearing is a critical factor in flatfish pigmentation development (Dhert *et al.*, 1994; Kanazawa, 1995; Rainuzzo *et al.*, 1997; Sargent *et al.*, 1997; Mangor *et al.*, 1998). Most studies have focused on two classes of nutrients: vitamin A and its precursors (Kanazawa, 1993; Dedi *et al.*, 1995; Estévez and Kanazawa, 1995; Takeuchi *et al.*, 1995, 1998; Dedi *et al.*, 1997; Rønnestad *et al.*, 1998a,b), and fatty acids, particularly DHA and EPA (Izquierdo *et al.*, 1992; Watanabe, 1993; Dhert *et al.*, 1994; Rainuzzo *et al.*, 1994, 1997; Estévez and Kanazawa, 1995; Sargent *et al.*, 1997, 1999a,b).

Detailed examinations of the biochemical composition of *Artemia* and other cultured live diets reveal that the levels and types of fatty acids and free amino acids differ significantly from those characteristic of wild zooplankton (Næss *et al.*, 1995; Sargent *et al.*, 1997). These differences are probably important factors in reducing pigmentation success in larvae fed exclusively on cultured diets. Although wild-caught prey form the optimal larval diet from a nutritional standpoint, the cost, seasonal availability and variation in species composition pose obstacles to their exclusive use in commercial hatchery production (Næss *et al.*, 1995; Gara *et al.*, 1998; Mangor *et al.*, 1998). Instead, aquaculturists have sought to replace wild zooplankton in larval diets with

cultured *Artemia* and rotifers. This approach offers greater reliability and control, but can also lead to high rates of malpigmentation.

Three strategies have been used to minimize malpigmentation in flatfish larvae fed cultured prey. The first is to select the strains of *Artemia* that cause the least malpigmentation (Seikai *et al.,* 1987a; Takeuchi *et al.,* 1995). The second approach is to rear larvae on cultured prey except during a relatively short period leading up to metamorphosis (Seikai and Sinoda, 1981; Seikai *et al.,* 1987a; Næss *et al.,* 1995; Næss and Lie, 1998). The third approach is to enrich prey organisms with specific compounds required by larvae, or with precursors from which larvae can synthesize essential compounds (Watanabe, 1993; Dhert *et al.,* 1994; Rainuzzo *et al.,* 1994, 1997; Kanazawa, 1995; Sargent *et al.,* 1977; Rønnestad *et al.,* 1998b). The last two approaches seem to offer the greatest control of larval diets and the best chance of developing long-term strategies to overcome malpigmentation, and have thus received the most attention (Sargent *et al.,* 1999b).

Enriching cultured prey can significantly enhance larval nutrition and pigmentation, and this technique is now commonly used in the rearing of flatfish and other marine species (Dhert *et al.,* 1994; Kanazawa, 1995; Rainuzzo *et al.,* 1997; Sargent *et al.,* 1997, 1999a,b). This method has been used both for fatty acids (Izquierdo *et al.,* 1992; Kanazawa, 1993; Watanabe, 1993; Rainuzzo *et al.,* 1994; Estévez and Kanazawa, 1995; Estévez *et al.,* 1997; Sargent *et al.,* 1997, 1999a; Baker *et al.,* 1998; McEvoy *et al.,* 1998) and for vitamin A compounds (Dedi *et al.,* 1995, 1997; Estévez and Kanazawa, 1995; Takeuchi *et al.,* 1995, 1998; Rønnestad *et al.,* 1998a,b).

1.1.4 Yellowtail flounder (*Limanda ferruginea*)

Interest in flatfish aquaculture is relatively high in Atlantic Canada. Besides Atlantic halibut (*Hippoglossus hippoglossus*), and from research done at the Ocean Sciences Centre, Memorial University of Newfoundland and elsewhere, other flatfish species, such as winter flounder (*Pleuronectes americanus*), witch flounder (*Glyptocephalus cynoglossus*), yellowtail flounder (*Limanda ferruginea*) and American plaice (*Hippoglossoides platessoides*), have also shown potential for aquaculture in this region. All these species occur in the waters of Atlantic Canada and have historically made an important contribution to the fisheries of the eastern seaboard (Litvak, 1994).

Yellowtail flounder is a small right-eyed flounder found in the East Coast of North America (Scott and Scott, 1988). Goff (1993) reported that yellowtail flounder has the highest fillet yield among small flatfish. The fillet yield from a 30-45 cm wild yellowtail flounder can be close to 40%. Thus, yellowtail flounder was chosen as a potential candidate for cold water aquaculture due to its high fillet-to-body ratio, its relatively high market price and a steadily declining wild stock size (Brown *et al.*, 1995; Brown and Crim, 1998; Brown, 2000). Yellowtail flounder was a commercially important species in the harvest fishery until the recent decline in stock abundance and there is every indication that it has good market potential (Brown *et al.*, 1995). Scott and Scott (1988) reported that yellowtail flounder has the fastest growth rate among the small commercial flounders. Larvae reach metamorphosis around 40 days post-hatch at 10°C. Boyce (2000) reported that survival of yellowtail flounder larvae through metamorphosis to settlement may reach > 50% with some egg batches and that mortality is low after

metamorphosis (<5%). Research on yellowtail larviculture has, however, focused on physical and biological aspects of this species (e.g., Murray *et al.*, 1994a,b; French, 1995; Puvanendran and Brown, 1995; Copeman, 1996; Murray *et al.*, 1996; Baglole *et al.*, 1997; Crim and Bettles, 1997; Larsson *et al.*, 1997; Morris, 1997; Boyce *et al.*, 1998; Clearwater and Crim, 1998; Manning and Crim, 1998; Benoit and Pepin, 1999a,b; Rabe, 1999; Richardson *et al.*, 1999; Boyce, 2000; Rabe and Brown, 2000; Avery, 2001). Currently, there is only one study that has been conducted on the nutritional aspect of larvae, particularly on the lipid nutrition, of this species (Copeman, 2001). As in other flatfish, high mortality and malpigmentation are the main challenge to the successful culture of yellowtail (Copeman *et al.*, 2002). Both of these issues have frequently been associated with the EFA contents of live feeds used in first feeding (Sargent *et al.*, 1999a,b).

1.2 Objectives

As part of on-going efforts to increase the use of alternative sources of n-3 PUFA in practical fish feeds, this study was designed to investigate the effects of various oil emulsions on live prey and their subsequent effects on larval fish fed on a diet supplemented with those oil emulsions in the hope of formulating novel feeds for aquaculture. My study should also expand our understanding of the role of dietary lipids with different characteristics in the nutritional value for cultured fish. Thus, the general objectives of my thesis were to investigate:

- how seal blubber oil, menhaden oil, Algamac and DHASCO affect the nutritional value of live feeds and their subsequent effects on performance (survival, growth, pigmentation and eye migration) of larval and juvenile yellowtail flounder.
- how seal blubber oil, menhaden oil, Algamac and DHASCO affect the lipids and fatty acid composition of live prey and of larval and juvenile yellowtail flounder.
- how enrichment concentrations and period of enrichment affect the lipids and fatty acid composition of live prey enriched with different oil emulsions.

The reasons for using seal oil in the present study were: a) seal oil is a byproduct of the sealing industry, and b) seal oil possesses unique chemical composition and structure characteristics that are different from those of fish oils. The n-3 HUFA (DHA, EPA and docosapentaenoic acid – DPA, 22:5n-3) in seal oils are located primarily in the *sn-1* and *sn-3* positions, while in fish oils they are located more abundantly in the *sn-2* position of the triacylglycerol (TAG) molecules.

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Chapter 2

Materials and Methods

2.1 Yellowtail flounder experiment

2.1.1 Rotifer enrichment

Newly harvested rotifers from a stock tank maintained at the Aquaculture Research and Development Facility, Ocean Sciences Centre, Memorial University of Newfoundland, were divided into 3 batches for enrichment with different sources of oil emulsions. Each batch was given an oil emulsion of either seal blubber oil (AMi Company, St. John's, NF), menhaden oil (Omega Protein, Reedville, VA) or Algamac-3010 (a trademark of Aquafauna Bio-Marine, Inc., Hawthorne, CA) in the first experiment, and seal oil, seal oil + DHASCO (DHA single cell oil; Martek Bioscience Corp., Columbia, MD) or Algamac-3010 in the second experiment. The use of seal oil in the second experiment was to verify its promising results, whereas that of Algamac was due to its unconvincing effect on survival of larvae in the first experiment. The Seal oil + DHASCO was used to determine if fortification of seal oil with DHA would convey better results on performance of flounder larvae. The fatty acid composition of oils used to prepare enrichment emulsions is presented in Table 2.1. The concentrations of seal oil (8.36% DHA) and menhaden oil (12.02% DHA) were 0.34 and 0.24 g per million of rotifers, respectively, 0.23 g for seal oil+DHASCO (12% DHASCO containing 42% DHA), whereas that of Algamac-3010 (26.8% DHA; lipid content 34.6%) was 0.3 g per million of rotifers. These concentrations provided approximately 28 mg of DHA for a million rotifers. The emulsions, after adjusting the amounts of enrichment media based

on the number of rotifers to be enriched, were prepared by vigorously blending each enrichment medium with 250 mL of UV-treated filtered seawater and 0.2 g raw egg yolk (~8–10 mg lipid/million rotifers, as emulsifier) for approximately 3 min.

Enrichment of rotifers was conducted 6 h post-harvest for 20 h in 12-L enrichment cones at 16-18°C. The density of rotifers in the enrichment media was 220/mL. All emulsions were divided into two portions; the second portion was offered 10 h after the first portion was given. At the end of the enrichment period, enriched rotifers were harvested through a 60-μm-mesh screen, washed thoroughly with filtered seawater to remove traces of oils, and subsequently fed to fish larvae. A small portion of enriched rotifers was taken (5 samplings at 4 days intervals) for lipid and fatty acid analyses. The fatty acid composition of unenriched rotifers used during the experiments is given in Appendix 2.2.

2.1.2 *Artemia* enrichment

Decapsulated *Artemia fransiscana* cysts (Premium *Artemia* cysts, Sea Dragon, Utah, USA) were incubated in illuminated, aerated seawater at 26-28°C and 30 ppt salinity for about 24 h before hatching. After hatching, nauplii were separated from empty cysts, washed and placed in filtered, aerated seawater. Newly hatched *Artemia* were divided into 3 batches for enrichment with different types of emulsion media. In the first experiment, each batch was given an oil emulsion of either seal blubber oil, menhaden oil or Algamac-3010, and seal oil, seal oil+DHASCO or Algamac-3010 in the second experiment. The concentrations of seal oil, menhaden oil and Algamac-3010 emulsions were 2.25, 1.56 and 2 g/million of nauplii, while that of seal oil+DHASCO

(4.21% DHASCO) was 1.90 g/million nauplii. These oil concentrations provided about 187.5 mg of DHA. Emulsions, after adjusting the amounts enrichment media based on the number of *Artemia* to be enriched, were prepared by vigorously blending each enrichment medium with 250 mL UV-treated filtered seawater and 0.4 g raw egg yolk (~16–20 mg lipid/million *Artemia*, as emulsifier) for approximately 3 min.

Enrichment of *Artemia* nauplii was conducted 6 h post-hatch in 12-L enrichment cones for 20 h at temperatures of 16-18°C. The density of nauplii in enrichment media was 125/mL. All emulsions were divided into two portions; the second portion was offered to *Artemia* 10 h after the first portion was given. At the end of the enrichment period, enriched nauplii were harvested through a 105-μm-mesh screen, washed thoroughly with filtered seawater to remove traces of oils, and subsequently fed to fish larvae. A small portion of enriched nauplii was taken (5 samplings at 5 days interval) for lipid and fatty acid analyses. The fatty acid composition of unenriched *Artemia* nauplii used during the experiments is given in Appendix 2.3.

2.1.3 Larval rearing and feeding

Eggs and milt of yellowtail flounder (*Limanda ferruginea*) were collected from captive broodstock maintained at the Ocean Sciences Centre, Memorial University of Newfoundland. Fertilized eggs were incubated in 300-L cylindro-conical upwelling tanks. Each incubation tank was supplied with an air stone and gentle aeration. The eggs hatched over a 24-h period after 5 days of incubation (65.7 degree days). Larvae were kept in incubators prior to transfer into experimental tanks on day 2, post-hatch.

In each experimental set-up, yellowtail flounder larvae were stocked into nine 90-L rectangular black plastic tanks (3 treatments with 3 replicates) at a density of 8 larvae/L. All tanks were kept in a water bath and supplied with filtered (25 μm) seawater in a flow-through system. Each tank was supplied with one air stone to provide gentle aeration as well as to promote homogenous distribution of the prey organisms. A 24-h light regime was used throughout the experimental period. The light was supplied by 4, 34-W fluorescent bulbs (General Electric) providing an intensity at the water surface of approximately 13.3 μmol s^{-1} m^{-1} (750 Lux) (Rabe and Brown, 2000). Water temperature ranged from 10.4 to 17.8°C (mean ± SD = 12.7 ± 1.7°C, n = 62) throughout the experiment.

The feeding experiment on yellowtail larvae was started on day 2-PFF (post first feeding), and this was considered as day-0. All treatments were fed enriched rotifers (*Brachionus plicatilis*) at a density of approximately 10 prey/mL until day 6-PFF. During this period, water flow was maintained at approximately 100 mL/min. From day 7 to 11, the rotifer density was increased to 20 prey/mL, and the water flow was subsequently adjusted to 200 mL/min. Day 12 to 20, the density of rotifers and the water flow rate were increased to 30 prey/mL and 300 mL/min, respectively. From day 21 to 27, the prey density was increased to 40 rotifers/mL and the water flow rate was adjusted to 400 mL/min. From day 28 to 40, water flow was maintained at 500 mL/min and then increased to 600 mL/min until the end of the experiment.

Co-feeding yellowtail larvae with rotifers and enriched *Artemia fransiscana* nauplii started on day 28 with prey densities of 30 rotifers and 2 *Artemia*/mL, respectively. While the density of rotifers was reduced to 20 prey/mL on day 31, that of

Artemia was increased to 5 nauplii/mL. On day 34, the rotifer regime was terminated, and the *Artemia* density was subsequently increased to 8 nauplii/mL. Finally, from day 40 to 50, and 51 to 62, the density of prey organisms was adjusted to 10 and 12 nauplii/mL, respectively. Following each feeding, approximately 2-L of algae (*Isochrysis galbana*) were added into each tank throughout the experimental period. All tanks were cleaned every 2 days by siphoning, while water temperature was measured daily between 11 am and 1 pm. Throughout the experimental period, all larvae were fed twice daily, which has been shown to be an adequate number of feedings (Rabe and Brown, 2000).

2.1.4 Sampling of fish larvae

Experimental larval fish were sampled on days 1, 8, 20 and 40 after the first feeding, and at the end of the experiment. Sampled larvae were measured for their length using a dissecting microscope (Wild M3 Heerbrugg, Switzerland), washed with distilled water and stored at −20°C until used for analysis. At the end of the experiment, the length of sampled juveniles was measured, incidences of albinism recorded and sufficient samples were taken for lipid and fatty acid composition analyses. The distribution of pigment on the juvenile fish body surfaces was identified following the modified scheme of Gara *et al.* (1998: Fig. 2.1). Gara's *et al.* original scheme was modified by adding two more pigmentation categories (category 2 and 3 in Fig. 2.1, based on observation in pigmentation patterns of experimental yellowtail flounder). Eye migration was classified for each individual according to the position of the blind side eye relative to the dorsal margin of the head using a four-point eye index scheme (Gara *et al.*, 1998: Fig. 2.2). The

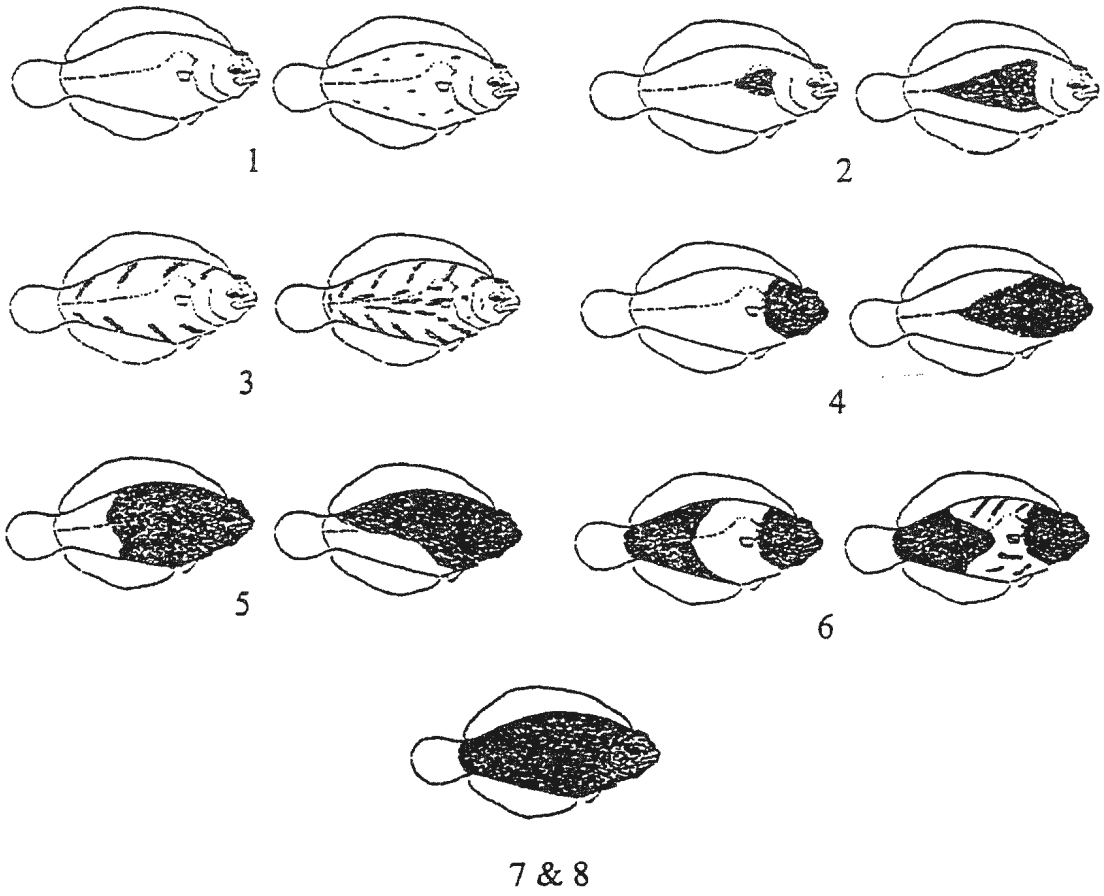

Fig. 2.1. Categories used to classify the pigmentation characteristics of yellowtail flounder (*Limanda ferruginea*). Category 7, complete pigmentation on the ocular side and no pigmentation on the blind side; Category 8, both sides are completely pigmented (modified from Gara *et al.*, 1998).

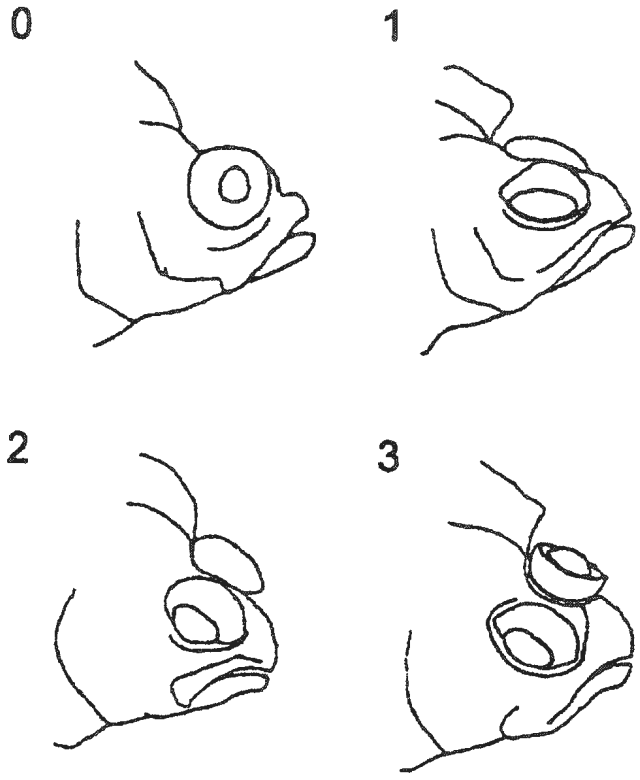

Fig. 2.2. Categories used to classify the eye migration characteristics of yellowtail flounder (*Limanda ferruginea*). 0, blind side eye not visible; 1, blind side eye just visible; 2, full diameter of blind side eye visible, but lens not visible; 3, complete eye migration with both eyes on the ocular side and lenses visible (After Gara *et al.*, 1998).

frequency of each pigment and eye migration categories were converted to a percentage of the whole population for each experimental tank. Samples of uneaten *Artemia* were also taken from each tank every 5-6 days in order to determine changes in their nutritional quality (decrease in essential fatty acids) after the de-enrichment period.

2.1.5 General performance of fish

Determination of general performance of fish in this study included survival, growth and pigmentation. Survival was calculated as the ratio of the number of fish surviving at the end of the experiment to the number of larvae stocked at the beginning of the experiment. The growth of the experimental fish was reported as specific growth rate and calculated as follows:

$$SGR = \ln lt2 - \ln lt1/t2 - t1 \times 100\%$$

where: lt2 is the length of fish at the end of the experiment, lt1 is the length of fish at the beginning of the experiment, t2-t1 is the duration of the experiment (day).

The pigmentation and eye migration type of fish were determined following categories described previously (section 2.1.4). The orientation of fish was determined at the end of the experiment and classified according to the side on which fish were lying and expressed as a percentage of the whole fish population for individual replicates.

2.1.6 Lipid analysis

2.1.6.1 Lipid extraction

2.1.6.1.1 Live feeds and larval fish

Lipids of rotifers, *Artemia* and larval fish were extracted with the chloroform-methanol-water solvent system (Bligh and Dyer, 1959) as modified by Işik *et al.* (1999). Approximately 0.5 – 1.0 g of rotifers or *Artemia* were weighed to the nearest 0.01 mg (Mettler AE-100; Mettler Instrumente AG, Greifensee, Zurich, Switzerland) into 25 mL test tubes and mixed with 6 mL of methanol containing 0.025% tert-butylhydroquinone (TBHQ; as an antioxidant). For larval fish, they were first crushed with a glass rod in a test tube before mixing them with methanol. The samples were then allowed to stand for 5 min with occasional stirring with a spatula. As much as 3 mL of chloroform and 2.2 mL of water were added and mixed using a vortex for approximately 30 s. The test tubes were then sonicated in an ultrasonic bath (Ultrasonik 300 NEY, Barkmeyer, Yucaipa, CA) at room temperature for 15 min. An additional 3 mL of chloroform and 3 mL of water were added, and the content of the test tubes was again mixed by vortexing for 30 s. The test tubes were then centrifuged (IEC Centra MP4, Needham Heights, MA) at 3500 x g for 15 min to separate the chloroform from the solids and the aqueous layers. After centrifugation, the methanol-water phase (upper layer) was withdrawn using a pasteur pipette and the chloroform phase (lower layer) containing lipids was transferred into a clean tube.

The solids left at the bottom of the test tubes were then re-extracted once more following the procedures described above and the chloroform from the first and the second extraction were combined. The lipid extracts were then passed through a 2.5 cm

thick layer of anhydrous sodium sulphate over a Whatman No.1 filter paper into a graduate cylinder, and the chloroform volume was subsequently recorded. The lipids were then dried by evaporating the solvent under reduced pressure in a Büchii rotary evaporator at 40°C. The dried lipids were recovered with 2 mL chloroform, transferred into 2 mL glass vials, flushed to dryness with nitrogen and stored at –20°C until used for analyses.

2.1.6.1.2 Juvenile fish

Lipids of juvenile fish were extracted into a chloroform-methanol-water solvent system according to Bligh and Dyer (1959) with slight modifications. A known weight of previously chopped samples (1 – 2 g) was first mixed with 10 mL methanol containing approximately 0.025% TBHQ in 50 mL centrifuge tubes and left to stand for about 10 min with occasional mixing. Then, 5 mL chloroform were added and the mixture homogenized for about 2 min using a Polytron PT 3000 (Brinkmann Instruments, Rexdale, ON) homogenizer. To the mixture, an additional 5 mL of chloroform were added, and after blending for about 30 s, 5 mL of distilled water was added followed by blending for another 30 s. The homogenized fish samples were then centrifuged (IEC Centra MP4, Needham Heights, MA) at 3500 x g for 15 min to separate the chloroform from the solids and the aqueous layers. After centrifugation, the methanol-water layer (upper phase) was withdrawn using a pasteur pipette and the chloroform layer (lower phase) containing lipids was transferred into a clean tube.

The slurry left at the bottom of the centrifuge tubes was then re-extracted twice as described above and the chloroform layers were combined. The lipid extracts were then

passed through 2.5 cm of anhydrous sodium sulphate over a Whatman No.1 filter paper into a graduated cylinder, and the chloroform volume was subsequently recorded. The lipids were then dried by evaporating the solvent under reduced pressure in a Büchii rotary evaporator at 40°C. The dried lipids were recovered with 2 mL chloroform, transferred into 2 mL glass vials, flushed to dryness with nitrogen and stored at –20°C until used for analyses.

2.1.6.2 Total lipid determination

The total lipid contents of live feeds and juvenile fish were determined gravimetrically. A known volume of the chloroform-containing lipids was transferred into a pre-weighed aluminum pan and the solvent was evaporated in a forced-air convection oven at 50°C. The dried matter was weighed and the content of total extracted lipid calculated.

2.1.6.3 Fatty acid composition of total lipid

Fatty acid composition of total lipids was determined using gas chromatograph (GC; HP-5890 Series II, Hewlett-Packard Ltd., Mississauga, ON). The lipid extract (5 – 10 mg) was transferred into 5 mL transmethylation vials and 3 mL of 6% H_2SO_4 (v/v) in 99.9 mole% methanol containing 0.01% mg TBHQ was added to the vials. The samples were then incubated overnight at 60°C in an oven. After incubation, 1.0 mL of distilled water was added to each vial and the mixture was extracted three times with 1.5 mL of pesticide-grade hexane. During the first extraction, a few more crystals of TBHQ were added. The hexane layer was transferred to a clean tube and then washed twice with 1.5

mL of distilled water by vortexing. At the first wash, the water layer was discarded; on the second wash, the hexane layer was transferred into a clean tube and evaporated under nitrogen. Dried matters were dissolved in 1.0 mL of carbon disulphide (CS_2) prior to GC analysis as described elsewhere (Shahidi and Dunajski, 1994; Metusalach *et al.,* 1995). A gas chromatograph equipped with a fused silica column (Supelcowax 10, 0.25 mm x 60 m, 0.25 μm film thickness; Supelco, Oakville, ON) and an HP-7673 GC/SFC autosampler and an HP-7673 controller/integrator were used for the analysis. The oven temperature was initially 40°C, ramped at 40°C/min up to a final temperature of 240°C and held there for 10 min. The injector and detector temperatures were set at 250°C from an initial temperature of 100°C. Helium was used as a carrier gas at a flow rate of 15 mL/min. The hydrogen pressure was set at 60 Psi while that of air was at 40 Psi. Individual fatty acids were identified by comparing their retention time with those of authentic fatty acid standards (Nu-Chek Prep. Inc., Elysian, MN).

2.2 Statistical Analysis

All experimental data on yellowtail flounder and live feeds used for larval rearing were subjected to one-way analysis of variance (one-way ANOVA; Minitab for Windows Release 13.20; Minitab Inc., State College, PA) to examine the effect of the test diets. One-way ANOVA was also used to compare the total lipid and fatty acid contents of the enriched rotifers and *Artemia* between enrichment concentrations, and between enrichment periods. When ANOVA indicated the existence of significant differences, Tukey's Test was employed to compare the individual mean value of parameters between the test diets at the 0.05 significance level. Simple regression analysis was used to

determine the type of relationship between n-3 HUFA, essential fatty acid (EFA) and EFA ratios in feeds and the rates of normal pigmentation (category 7+8), complete eye migration (category 3) and complete pigmentation + complete eye migration of the flounder larvae. Since the onset of metamorphosis, including pigmentation and eye migration, occurs within four weeks post-hatch (Copeman *et al.,* 2002), determination of this relationship was carried out using only the n-3 HUFA, EFA and EFA ratios in enriched rotifers. Two-way analysis of variance (two-way ANOVA) was employed to examine the effect of enrichment period and emulsion concentration on the total lipid, DHA, EPA and AA contents of rotifers and *Artemia*. Multiple linear regression was employed to determine the type of relationship existing between enrichment period and emulsion concentration and the total lipid and essential fatty acid (EFA) contents of the enriched rotifers and *Artemia*. All percentage data were arcsine transformed and tested for their normality and homogeneity before being subjected to an appropriate statistical analysis. Graphs were produced using SigmaPlot 2000 for windows (SPSS Science, SPSS Inc., Chicago, IL).

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Chapter 3

Effect of Live Feeds Enriched with Different Oil Emulsions on Survival, Growth, Pigmentation and Eye Migration of Yellowtail Flounder (*Limanda ferruginea*)

3.1 Introduction

It has been more than two decades since the start of flatfish culture in Europe, however, low rates of survival and high occurrences of malpigmented juveniles are still major problems faced by the aquaculture industry (Estévez *et al.*, 1999). Marine fish larvae are in general small, delicate, rapidly developing and growing organisms, which are highly susceptible to environmental stress (Bell, 1998). High larval mortality usually occurs around the period of first feeding when larval endogenous reserves are depleted. Both high mortality and malpigmentation have mainly been associated with the lack of nutritionally adequate live prey used in larval rearing. To reduce mortality and ensure normal growth and development, nutritional composition must be optimized both qualitatively and quantitatively. Much research has been directed toward determining the optimal feeding strategies and nutritional requirements for marine flatfish larvae, and considerable advances have been made (Bell, 1998).

Good survival and high rates of normal pigmentation have been demonstrated for Japanese flounder, *Paralichthys olivaceus* (Seikai, 1985) and halibut, *Hippoglossus hippoglossus* (Næss *et al.*, 1995) using wild zooplankton as live prey for fish larvae. The use of wild zooplankton is, however, limited by seasonal fluctuations in its availability

together with mismatches between the size of available copepods and the requirements of the fish larvae (Næss, 1996). Enriching live prey, such as rotifers (*Brachionus plicatiles*) and brine shrimp (*Artemia* sp.), with n-3 polyunsaturated fatty acids (PUFAs), phospholipids and vitamin A has also been successful in improving pigmentation in several species of flatfish (Miki *et al.*, 1990a,b; Kanazawa, 1993; Reitan *et al.*, 1994; Estévez and Kanazawa, 1995). Consequently, considerable research effort has been directed towards determining the lipid nutritional requirements of marine fish larvae, despite the difficulties involved in rearing such small and vulnerable animals (Bell, 1998).

The brine shrimp, *Artemia*, and the rotifers, *Brachionus plicatilis*, are commonly used as live prey, but have resulted in poor larval growth and survival (Skjolddal *et al.*, 1990). *Artemia* and rotifers are also known to cause a high proportion of abnormally pigmented larvae in flatfish (Watanabe *et al.*, 1978; Seikai *et al.*, 1987a,b). These effects are considered to be caused by the absence or deficiency of certain essential nutrients, particularly the PUFAs (Léger *et al.*, 1986; Seikai *et al.*, 1987a,b; Watanabe, 1993; Whyte *et al.*, 1994; Næss *et al.*, 1995). Although pigmentation abnormalities have no pathogenic effect on the fish (Soutar, 1995), chances of survival in the wild as well as the commercial value of abnormally pigmented fish are greatly reduced (Seikai and Matsumoto, 1991; Næss and Lie, 1998; Venizelos and Benetti, 1999). Malpigmentation of cultured flatfish juveniles continues to be a major problem for mariculturists due to its irreversible nature (Næss and Lie, 1998). The mechanism of pigmentation abnormalities and prevention methods are still unknown. It seems that the mechanism controlling the pigmentation in the early stages of flatfish is development-dependent (Seikai *et al.*,

1987c), but little information exists for the different species. Two hypotheses have been suggested to explain the origin of pigmentation problems (Estévez, 1996): changes in some components of the skin prior to the differentiation of pigment cells or changes in the normal composition of the retina and the brain due to dietary deficiencies.

Significant progress has been achieved in the biochemical manipulation of live prey (Planas and Cunha, 1999), and most attention has been paid to lipids and n-3 highly unsaturated fatty acids (HUFAs). It is well established that rotifers and *Artemia* composition reflects the biochemical composition of their diet, particularly that of lipids. These n-3 HUFA are found in both triacylglycerols and phospholipids, and while the former are used mostly as an energy source, the latter are the major component of cellular membranes. The unusual richness of docosahexaenoic acid (DHA, 22:6n-3) in the membranes of cells of the nervous system suggests that this fatty acid plays a critical role in the formation of the brain and eyes, which constitute a large fraction of the larval biomass (Howel *et al.*, 1998). Owing to its role in the formation of the retina, DHA also plays an important role in the development of normal pigmentation in flatfish, as production of melanin is stimulated by a hormone secreted in response to visual information being transmitted to the brain (Kanazawa, 1993). While less attention has been paid to the role of eicosapentaenoic acid (EPA, 20:5n-3), the ratio of DHA/EPA is considered to play an important role in healthy development (Rodriguez *et al.*, 1994), and has been found to modulate the dietary requirement for DHA in relation to growth and pigmentation in turbot (Reitan *et al.*, 1994). Watanabe *et al.* (1989a,b) reported that the nutritive value of EPA as an essential fatty acid might be inferior to DHA. EPA also

modulates by inhibition of the formation of eicosanoids from arachidonic acid (AA, 20:4n-6), which are produced in response to a range of external stressors (Sargent, 1995).

It is well documented that marine fish species require preformed EPA and DHA, i.e., they lack the ability to chain elongate 18:3n-3 PUFA. Because of this inability, n-3 HUFA should be present in the diet in sufficient quantities for healthy larval development. Dietary n-3 HUFA supplementation of live prey has profound effects, expressed as increased larval survival and increased stress tolerance. Tuncer *et al.* (1993) reported an increase in shock syndrome (handling stress) and a decrease in post-harvest survival, growth and size of palmetto bass larvae (*M. saxatilis* x *M. chrysops*) with decreasing n-3 HUFA in their diets. These authors also reported that conversion of short chain n-3 series to n-3 HUFA was not evident and that dietary supplementation was necessary for increased growth and survival in intensive larval culture of palmetto bass.

Preferential conservation of n-3 HUFAs in starved larval fish indicates the key roles played by these fatty acids. Conservation of n-3 HUFAs has been shown for striped bass (Martin *et al.*, 1984), gilthead seabream, *Sparus aurata* (Koven *et al.,* 1989) and palmetto bass, *M. saxatilis x M. chrysops* (Tuncer *et al.,* 1993). Koven *et al.* (1989) suggested that n-3 HUFA was conserved in starved larvae in order to maintain structural integrity of cell membranes. A rapid decrease in body n-3 HUFA in palmetto bass was prominent specifically during the early larval period (4-9 days old) when dietary intake of n-3 HUFA was in trace amounts. A continued decline in body n-3 HUFA content was evident in fish fed low n-3 HUFA diets (Tuncer *et al.,* 1993). Koven *et al.* (1989) also found rapid utilization of n-3 HUFA in feeding fish, which was attributed to intense tissue growth during fast larval growth. Koven *et al.* (1989) found that DHA was utilized

faster in feeding larvae and selectively more conserved in starved larvae than EPA. Likewise, DHA in fish was utilized faster than EPA during larval development. It is, therefore, important that the nutritional value of live prey is satisfactory by the time it is offered to larval fish.

To date, there is only one study addressing lipid nutrition in yellowtail flounder (*Limanda ferruginea*) larvae. Copeman *et al.* (2002), using specially prepared oil emulsions containing high levels of essential fatty acids (EFAs) to enrich rotifers, fed larval yellowtail flounder for four weeks and found that this species requires high levels of dietary DHA for maximal growth and survival. They also reported that high level of arachidonic acid (AA) produced a negative effect on pigmentation. More research is needed for a better understanding about lipid nutrition and the role of essential fatty acids in this species. The present study was aimed at comparing different oil emulsions for their efficacy to enrich rotifers and *Artemia* with essential fatty acids and their subsequent effects on yellowtail flounder larvae.

3.2 Materials and methods

Refer to Chapter 2 section 2.1, sub-section 2.1.1 to 2.1.5.

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Table 3.1. Gape (%), survival rate (%), specific growth rates (%/day), rearing temperature (°C), pigmentation, eye migration and orientation of yellowtail flounder (*Limanda ferruginea*) fed live prey enriched with seal oil, seal oil+DHASCO, menhaden oil and Algamac-3010 over a 62-day period.

Parameters		First experiment						Second experiment					
		Seal oil		Menhaden oil		Algamac-3010		Seal oil		Seal oil+DHASCO		Algamac-3010	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Gape				28.3 ± 2.1[a]						36.3 ± 2.6[b]			
Survival		20.4[a]	5.1	16.1[ab]	0.1	11.1[b]	1.9	9.9[x]	1.6	6.5[y]	2.2	5.7	0.5
Growth rate (%/day)		3.50[a]	0.01	3.53[ab]	0.02	3.69[b]	0.03	3.26	0.16	3.29	0.17	3.32	0.15
Temperature (°C)		12.7	1.7	12.7	1.7	12.7	1.7	13.2	1.4	13.2	1.4	13.2	1.4
Pigmentation Type:	1	21.3	3.2	25.6	5.0	25.4	2.7	1.3[x]	0.2	18.7[y]	4.1	7.1[z]	2.1
	2	5.0[a]	1.6	0.5[b]	0.1	5.2[a]	1.5	1.3	0.4	1.2	0.3	-	-
	3	24.3[a]	1.9	18.7[ab]	4.0	15.4[b]	3.1	50.5	7.3	36.6	6.2	41.8	6.4
	4	0.3[a]	0.1	0.5[ab]	0.1	0.8[b]	0.2	-	-	-	-	-	-
	5	4.2[a]	1.4	1.5[b]	0.5	6.0[a]	0.6	-	-	-	-	-	-
	6	1.6	1.4	-	-	1.2	0.4	-	-	-	-	0.3	0.2
	7	27.4[a]	3.7	39.9[b]	6.2	40.5[b]	2.1	40.0	1.1	35.3	2.4	38.4	6.4
	8	15.7[a]	2.5	13.2[a]	0.7	5.8[b]	2.4	7.5	3.5	8.3	3.0	12.1	2.2
Eye migration type:	0	20.8[a]	2.5	19.2[a]	3.5	10.0[b]	1.8	20.5[x]	3.6	14.4[xy]	3.7	9.6[y]	1.6
	1	15.7[a]	1.2	13.3[a]	1.5	7.9[b]	2.4	8.8	1.3	10.6	2.2	12.2	2.1
	2	7.2[ab]	1.1	5.8[a]	1.9	9.6[b]	2.1	12.5	2.3	13.4	2.8	11.8	2.2
	3	55.6[a]	6.4	61.7[a]	4.0	72.4[b]	4.8	58.3	7.5	61.6	6.3	66.4	6.4
CP & CEM**		31.0[a]	0.74	44.8[b]	4.21	41.1[b]	1.5	26.3	3.8	24.8	3.8	27.9	7.9
Orientation:	Right	75.0	3.4	67.5	2.3	71.8	3.0	79.3	5.3	81.3	6.3	80.7	4.3
	Left	25.0	3.4	32.5	2.3	28.1	3.0	19.3	5.3	18.8	6.3	19.3	4.3

Values are means and standard deviations (SD) of 3 replicates. *Complete pigmentation and complete eye migration. Type-8 of pigmentation is an ambicolouration (pigmented on both sides).

Values in each row under each experimental set-up with different superscripts (a,b or x,y) are different ($p<0.05$) from one another. No superscript indicates the absence of significant difference for each experimental set-up.

3.3.2 Growth

The initial sizes of yellowtail flounder larvae used in the first and second experiments were similar (3.6 and 3.5 mm, respectively). After 62 days of feeding, flounder juveniles in the first experiment reached an average length of 31.3, 31.9 and 35.2 mm for seal oil, menhaden oil and Algamac treatments, respectively. In the second experiment, the average length of juveniles at the end of the experiment was 26.7, 27.2 and 27.7 mm for seal oil, seal oil+DHASCO and Algamac treatments, respectively. The size of the experimental fish was not different ($p>0.05$) among treatments for each experimental setup (Fig. 3.1). In the second experiment, there was a mass mortality 14-16 days post first feeding (personal observation), and it appeared that larger larvae died. This was apparent from the size of larvae sampled 20 days after the onset of feeding trials where larval size remained similar to that of larvae sampled 12 days earlier.

The growth rates of flounder larvae decreased throughout the experimental period (Fig. 3.2). In the first experiment, the growth of flounder larvae was higher ($p<0.05$) in menhaden oil treatment compared to the other treatments during the early feeding period (8 days post first feeding), while larvae in the Algamac treatment exhibited a higher ($p<0.05$) growth rate at the end of the experiment compared to the growth rate of larvae fed seal oil. In the second experiment, larval flounder from all treatments showed similar growth rates on each sampling date. The lower growth rates at 20 days post first feeding in the second experiment were a direct result of the larger larvae dying from the population. At the end of the experiment, the growth rates of larvae in the first experiment were 3.50, 3.53 and 3.69%/day for seal oil, menhaden oil and Algamac treatments, respectively, whereas those of flounder larvae in the second experiment were

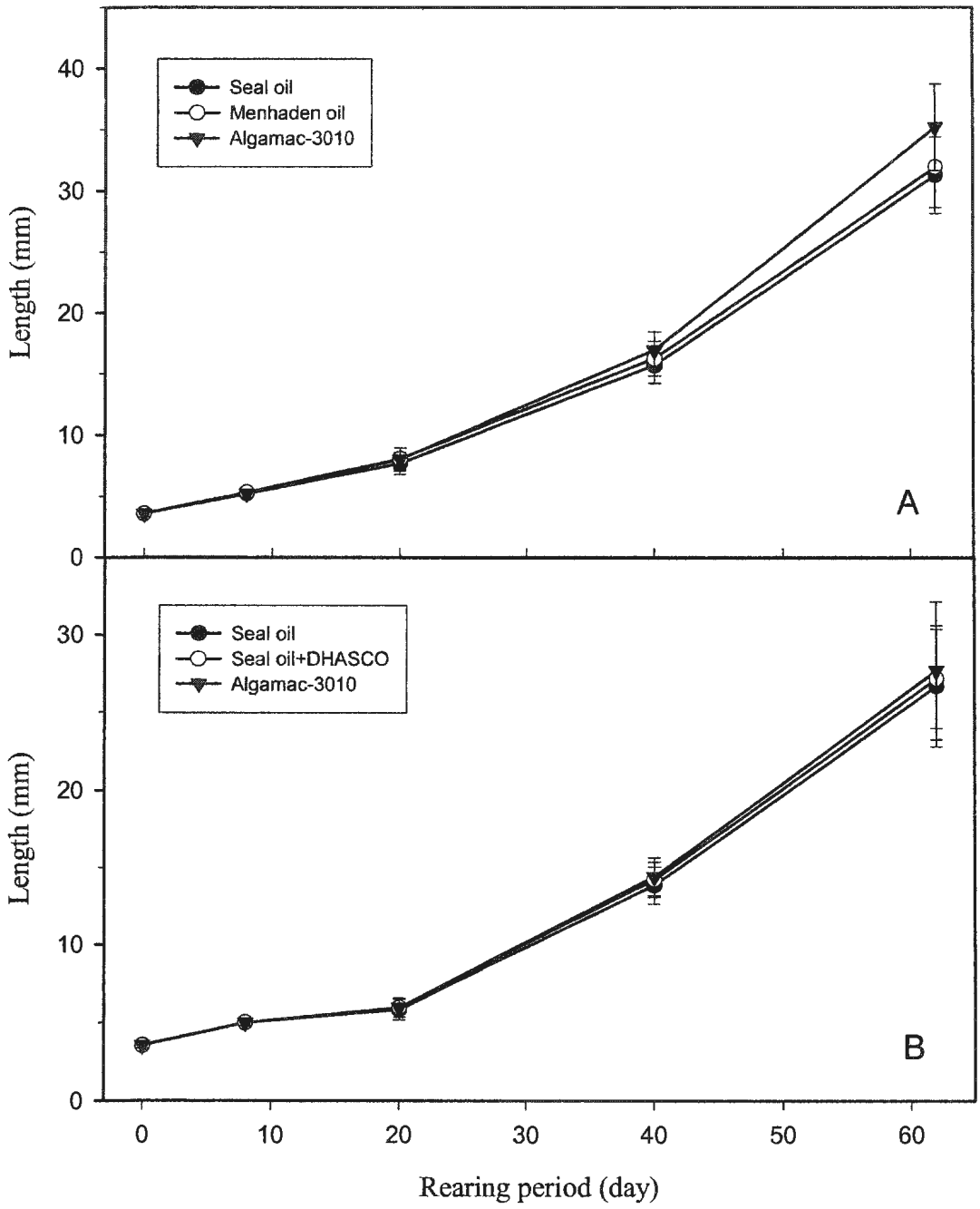

Fig. 3.1. Size (mm) of yellowtail flounder (*Limanda ferruginea*) larvae fed live feeds enriched with different oil emulsions. A and B - the first and second experiment, respectively. Vertical bars represent standard deviations, n = 24-238.

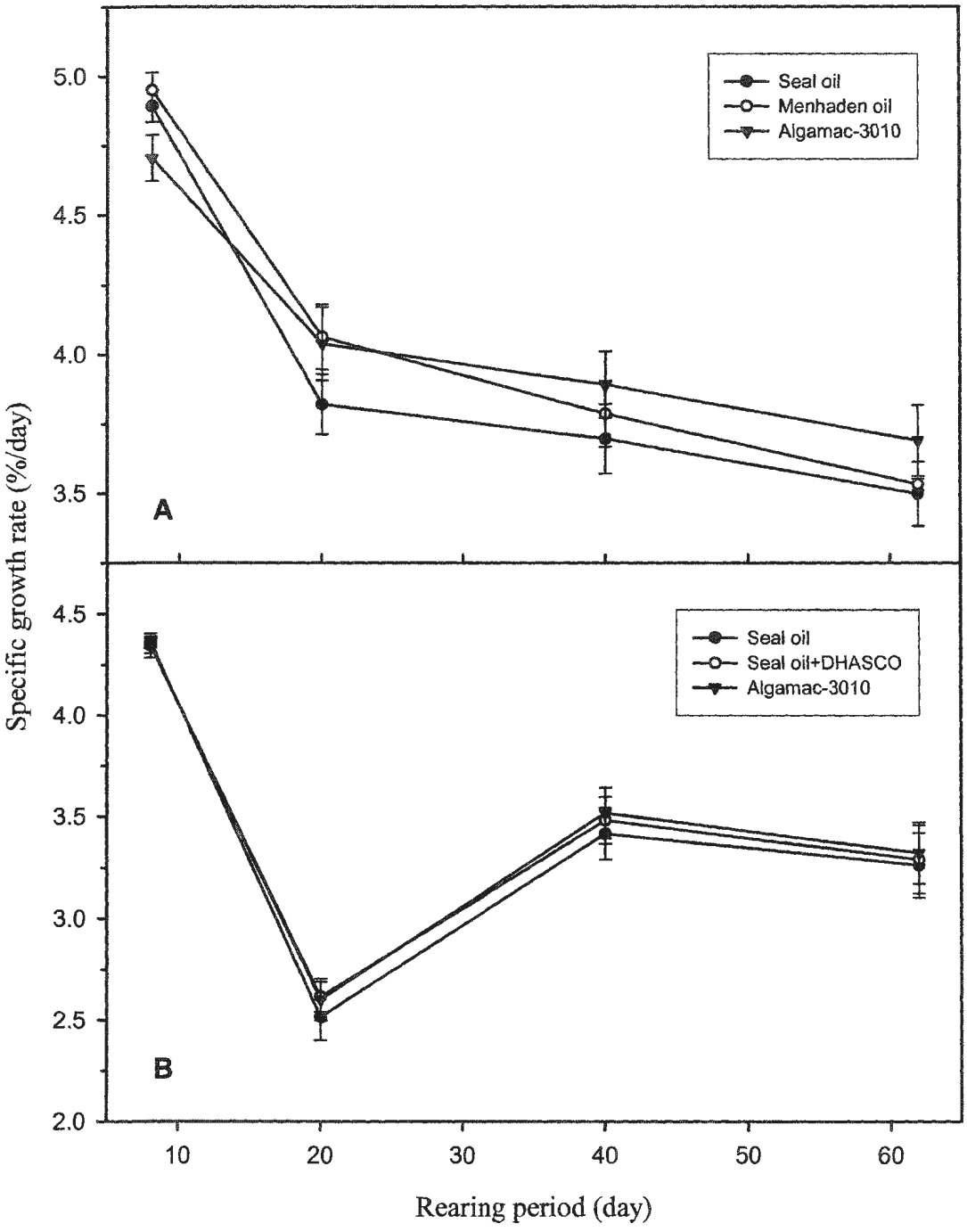

Fig. 3.2. Specific growth rates of yellowtail flounder (*Limanda ferruginea*) larvae fed live feeds enriched with different oil emulsions. A and B - the first and second experiment, respectively. Vertical bars repredent standard deviations, n = 3.

3.26, 3.29 and 3.32%/day for seal oil, seal oil+DHASCO and Algamac treatments, respectively.

3.3.3 Pigmentation, eye migration and orientation

The proportion of each pigmentation category of yellowtail flounder fed enriched live feeds is presented in Table 3.1. In the first experiment, there were 8 categories of pigmentation (Chapter 2; Fig. 2.1) observed, whereas only 6 categories were found in the second experiment. Categories 7 (27 – 40%), 1 (21 – 25%) and 3 (15 – 24%) were the most dominant pigmentation categories found in the first experiment. Category 8 of pigmentation (ambicolouration, pigmented on both sides) was also present at considerably high proportions (up to 16%), while the proportion of category 2, 4, 5 and 6 were much lower (less than 6%). In the second experiment, however, categories 3 and 5 were more prevalent (36 – 50 and 35 – 40%, respectively). Category 8 accounted for relatively high proportions (7 – 12%). The proportion of fish that underwent complete pigmentation (category 7 + 8) ranged from 43 to 53% in the first experiment, and from 44 to 51% in the second experiment (Fig. 3.3).

Eye migration was differentiated into 4 categories (Chapter 2, Fig. 2.2). Category 3 of eye migration (complete migration) accounted for the highest proportion of the experimental fish (56 to 72% in the first experiment and 58 to 66% in the second experiment, Table 3.1). In both experimental setups, fish in the Algamac feeding exhibited the highest proportion of complete eye migration and it was lowest in fish fed seal oil. Significant ($p<0.05$) differences among the feeding treatments were, however, observed only in the first experiment.

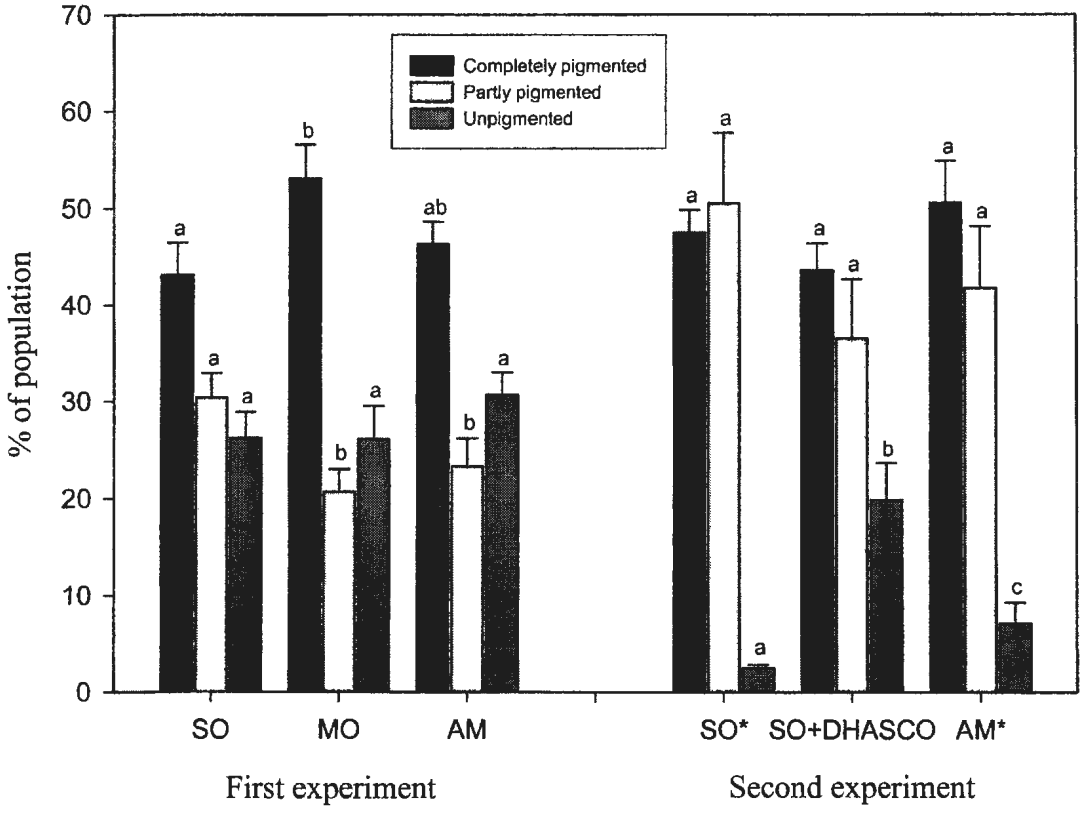

Fig. 3.3 Pigmentation success in yellowtail flounder (*Limanda ferruginea*) fed live feeds enriched with various oil emulsions. Vertical bars represent standard deviations, n = 3. Different letters (a,b) indicate significant difference at 0.05 level.

One of the criteria in determining good quality flatfish juveniles is complete pigmentation and eye migration. In this experiment, the proportion of flounder juveniles that had complete pigmentation and eye migration varied ($p<0.05$) among treatments in the first experiment (Table 3.1). Yellowtail flounder juveniles that had complete pigmentation and eye migration accounted for up to 45% of the juveniles in menhaden oil feeding, 41% in Algamac feeding and 31% in seal oil feeding. In the second experiment, however, the proportion of fish that underwent complete pigmentation and eye migration was similar among feeding treatments and was much lower (26, 25 and 28% for seal oil, seal oil+DHASCO and Algamac treatments, respectively) compared to values in the first experiment.

The majority of flounder juveniles in this experiment were "right-oriented" or "right-sided" (fish lie on left side) (Table 3.1). The proportion of right-oriented fish ranged from 67% in the first experiment to 81% in the second experiment. For each experimental setup, neither the proportion of right-oriented nor left-oriented fish showed any significant ($p>0.05$) difference among treatments.

3.3.4 The contents of n-3 HUFA and EFA and EFA ratios in enriched live feeds used during the yellowtail flounder experiments

The contents of n-3 HUFA and essential fatty acids of enriched and uneaten live feeds used in this experiment are presented in Table 3.2, 3.3 and 3.4. In rotifers, the n-3 HUFA contents constituted 17.9 to 30.6% (3.2 – 5.5% diets dry wt.) of total fatty acids. DHA was the most abundant EFA in this prey regardless of the enrichment media, followed by EPA. Meanwhile, AA was found at much smaller amounts, ranging from

Table 3.2. Proportions of saturated, monounsaturated, polyunsaturated, n-3 highly unsaturated and essential fatty acids of live prey used in the first experiment with yellowtail flounder (*Limanda ferruginea*).

Fatty acid	Live prey enriched with: (first experiment)					
	Seal oil		Menhaden oil		Algamac-3010	
	% FA	% DW	% FA	% DW	% FA	% DW
Rotifers						
SFA	13.3^{a}	2.26^{x}	21.4^{b}	3.59^{y}	21.9^{b}	3.87^{y}
MUFA	55.0^{a}	9.32^{x}	43.8^{b}	7.37^{y}	34.7^{c}	6.14^{z}
PUFA	26.3^{a}	4.46^{x}	27.7^{a}	4.66^{x}	37.7^{b}	6.68^{y}
n-3 HUFA	18.7^{a}	3.16^{x}	18.7^{a}	3.15^{x}	27.7^{b}	4.90^{y}
DHA	7.09^{a}	1.20^{x}	6.62^{a}	1.11^{x}	22.3^{b}	3.95^{y}
EPA	5.90^{a}	1.00^{x}	6.49^{b}	1.09^{x}	3.70^{c}	0.65^{y}
AA	1.35^{a}	0.23^{x}	1.68^{b}	0.28^{x}	2.29^{c}	0.41^{y}
DHA/EPA	1.20^{a}		1.02^{a}		6.04^{b}	
EPA/AA	4.42^{a}		3.87^{a}		1.61^{b}	
DHA/AA	5.32^{a}		3.94^{a}		9.72^{b}	
Artemia						
SFA	18.9^{a}	3.49^{x}	20.1^{a}	3.59^{x}	18.2^{a}	3.78^{x}
MUFA	39.0^{a}	7.20^{x}	35.2^{b}	6.28^{y}	25.0^{c}	5.19^{z}
PUFA	42.7^{a}	7.89^{x}	46.5^{b}	8.32^{x}	56.9^{c}	11.8^{y}
n-3 HUFA	11.7^{a}	2.16^{x}	10.0^{a}	1.79^{x}	27.0^{b}	5.61^{y}
DHA	2.65^{a}	0.49^{x}	3.50^{b}	0.63^{y}	15.2^{c}	3.16^{z}
EPA	7.79^{a}	1.44^{x}	6.53^{ab}	1.17^{y}	6.43^{b}	1.33^{z}
AA	1.02^{a}	0.19^{x}	1.10^{a}	0.20^{x}	2.93^{b}	0.61^{y}
DHA/EPA	0.35^{a}		0.48^{a}		2.37^{b}	
EPA/AA	7.62^{a}		5.95^{b}		2.19^{c}	
DHA/AA	2.59^{a}		3.19^{b}		5.19^{c}	

Analyses were carried out in 5 replicates. Typical coefficient of variation is ~5%. FA – fatty acid; DW – dry weight.

Values in each row with the same superscripts (a,b,c or x,y,z) are significantly ($p<0.05$) different from one another.

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; HUFA, highly unsaturated fatty acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Table 3.3. Proportions of saturated, monounsaturated, polyunsaturated, n-3 highly unsaturated and essential fatty acids of live prey used in the second experiment with yellowtail flounder (*Limanda ferruginea*).

Fatty acid	Live prey enriched with: (second experiment)					
	Seal oil		Seal oil + DHASCO		Algamac-3010	
	% FA	% DW	% FA	% DW	% FA	% DW
Rotifers						
SFA	11.9^{a}	2.09^{x}	13.7^{a}	2.29^{x}	27.4^{b}	4.94^{y}
MUFA	53.8^{a}	9.50^{x}	49.9^{a}	8.32^{y}	26.1^{b}	4.71^{z}
PUFA	28.3^{a}	4.99^{x}	28.9^{a}	4.81^{x}	39.2^{b}	7.07^{y}
n-3 HUFA	17.9^{a}	3.15^{x}	18.9^{a}	3.16^{x}	30.6^{b}	5.52^{y}
DHA	6.86^{a}	1.21^{x}	11.2^{b}	1.86^{y}	24.8^{c}	4.48^{z}
EPA	5.83^{a}	1.03^{x}	3.89^{b}	0.65^{y}	3.10^{c}	0.56^{y}
AA	0.76^{a}	0.13^{x}	0.54^{b}	0.09^{x}	1.65^{c}	0.30^{y}
DHA/EPA	1.18^{a}		2.88^{b}		8.02^{c}	
EPA/AA	7.66^{a}		7.23^{a}		1.87^{b}	
DHA/AA	9.02^{a}		20.8^{b}		15.0^{c}	
Artemia						
SFA	21.1^{a}	3.63^{x}	22.5^{a}	4.08^{y}	20.5^{a}	3.95^{y}
MUFA	38.6^{a}	6.63^{x}	34.9^{b}	6.33^{xy}	32.0^{b}	6.19^{y}
PUFA	34.7^{a}	5.96^{x}	36.6^{a}	6.63^{x}	43.8^{b}	8.46^{y}
n-3 HUFA	9.02^{a}	1.55^{x}	12.0^{b}	2.17^{y}	18.0^{c}	3.47^{z}
DHA	2.22^{a}	0.38^{x}	5.31^{b}	0.96^{y}	12.0^{c}	2.32^{z}
EPA	4.17^{a}	0.72^{x}	4.85^{b}	0.88^{y}	4.13^{a}	0.80^{z}
AA	0.69^{a}	0.12^{x}	0.61^{b}	0.11^{x}	0.79^{c}	0.15^{y}
DHA/EPA	0.53^{a}		1.09^{b}		2.91^{c}	
EPA/AA	6.06^{a}		7.95^{b}		5.20^{c}	
DHA/AA	3.22^{a}		8.71^{b}		15.2^{c}	

Analyses were carried out in 5 replicates. Typical coefficient of variation is ~5%. FA – fatty acid; DW – dry weight.

Values in each row with the same superscripts (a,b,c or x,y,z) are different ($p<0.05$) from one another.

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; HUFA, highly unsaturated fatty acid; DHASCO, DHA-rich algal oil; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Table 3.4. Proportions of saturated, monounsaturated, polyunsaturated, n-3 HUFA and essential fatty acid of uneaten *Artemia* during yellowtail flounder (*Limanda ferruginea*) experiments.

Fatty acid	*Artemia* enriched with: (first experiment)					
	Seal oil		Menhaden oil		Algamac-3010	
	% FA	% DW	% FA	% DW	% FA	% DW
SFA	19.0^{a}	2.93^{x}	23.3^{b}	3.56^{y}	17.9^{a}	3.01^{x}
MUFA	41.5^{a}	6.40^{x}	34.1^{b}	5.21^{y}	25.8^{c}	4.34^{z}
PUFA	38.7^{a}	5.97^{x}	41.7^{a}	6.37^{y}	55.6^{b}	9.36^{z}
n-3 HUFA	10.4^{a}	1.61^{x}	10.8^{a}	1.65^{x}	27.0^{b}	4.54^{y}
DHA	0.32^{a}	0.05^{x}	0.86^{b}	0.13^{x}	11.5^{c}	1.94^{y}
EPA	8.16^{a}	1.26^{x}	6.72^{b}	1.03^{y}	8.53^{a}	1.44^{z}
AA	0.43^{a}	0.07^{x}	0.46^{a}	0.07^{x}	3.95^{b}	0.66^{y}
DHA/EPA	0.04^{a}		0.13^{b}		1.36^{c}	
EPA/AA	19.1^{a}		14.8^{b}		2.16^{c}	
DHA/AA	0.75^{a}		1.89^{b}		2.93^{c}	

Analyses were carried out in 5 replicates. Typical coefficient of variation is ~5%. FA – fatty acid; DW – dry weight.

Values in each row with the same superscripts (a,b,c,d, or x,y,z) are different ($p<0.05$) from one another.

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; HUFA, highly unsaturated fatty acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

0.54% in the seal oil+DHASCO to 2.29% in the Algamac treatment. The reverse is, however, true in *Artemia* enriched with seal oil, menhaden oil and seal oil+DHASCO, where EPA existed as the most abundant EFA; the DHA, however, remained as the dominating EFA in Algamac-enriched *Artemia*. AA accounted for 0.69 to 2.93% of the fatty acids. The n-3 HUFA contents in *Artemia* ranged from 9.02 to 29.5% (1.55 – 5.61% diets dry wt.) of total fatty acids. AA existed at much lower levels (~ 50% less) in the prey used in the second experiment as compared to those in the first experiment. The contents of EFAs and EFA ratios (DHA/EPA, EPA/AA and DHA/AA) in prey were different ($p<0.05$) according to the enrichment media (Fig. 3.4 and 3.5). The detailed fatty acid composition of enriched live feeds can be seen in Appendix 3.1 – 3.4).

3.3.5 Relationship between n–3 HUFA, EFA contents and EFA ratios in rotifers and pigmentation and eye migration of yellowtail flounder

Table 3.5 presents the correlation coefficients between n-3 HUFA, EFA contents and EFA ratios in rotifers and pigmentation success (complete pigmentation, category 7+8) as well as eye migration of yellowtail flounder juveniles. In the first experiment, the n-3 HUFA, DHA, EPA and AA contents and DHA/AA ratio in rotifers did not correlate with complete pigmentation ($r = -0.32$ to 0.39; $p = 0.30 - 0.78$) and complete pigmentation + complete eye migration ($r = -0.37 - 0.54$; $p = 0.13 - 0.95$) of the juvenile flounder. However, the n-3 HUFA, DHA and AA contents as well as the DHA/EPA and DHA/AA ratios positively correlated with the eye migration ($r = 0.78 - 0.94$; $p < 0.001 - p = 0.048$) of the juvenile fish. On the other hand, the EPA content and EPA/AA ratio

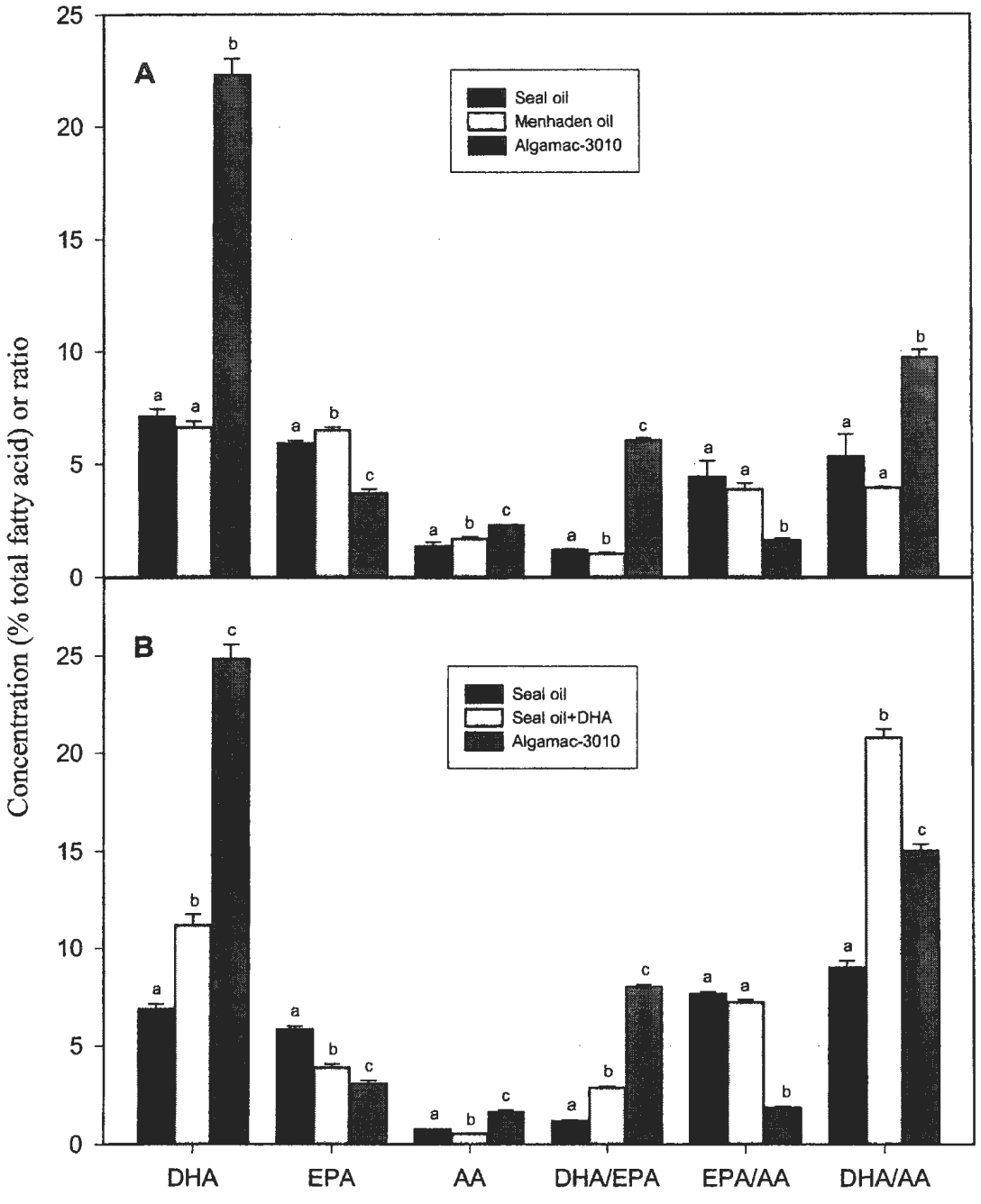

Fig. 3.4. Concentration of DHA, EPA and AA, and their respective ratios in the total lipid of rotifers (*Brachionus plicatilis*) enriched with different oil emulsions used to feed yellowtail (*Limanda ferruginea*) larvae. A and B - the first and second experiments, respectively. Vertical bars represent standard deviations, n = 5. Different letters (a,b,c) represent significant differences at 0.05 level.

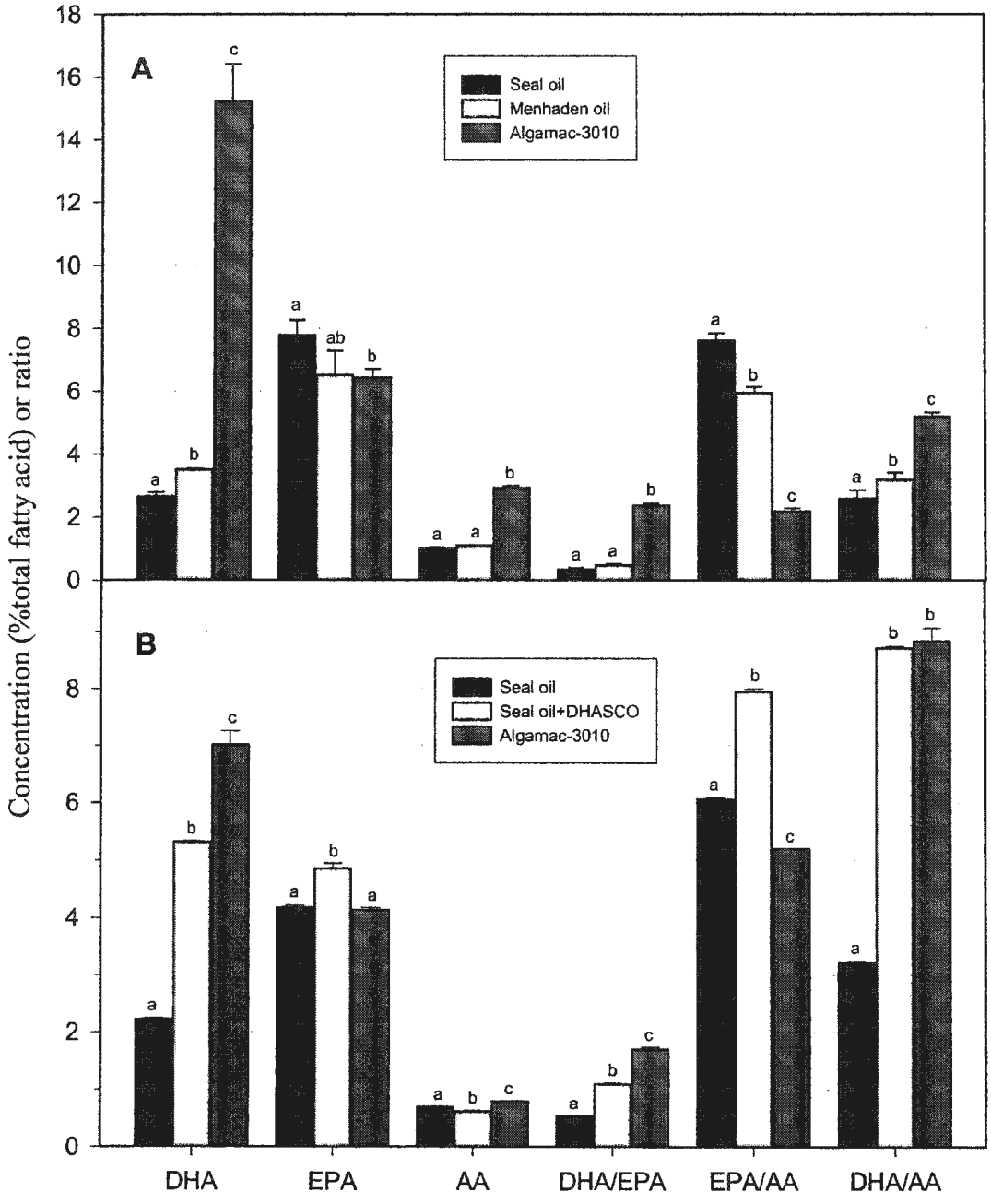

Fig. 3.5. Concentration of DHA, EPA and AA, and their respective ratios in the lipid of *Artemia* enriched with different oil emulsion used to feed yellowtail flounder (*Limanda ferruginea*) larvae. A and B - the first and second experiments, respectively. Vertical bars represent standard deviations, n = 5. Different letters (a,b,c) represent significant differences at 0.05 level.

Table 3.5. Correlation coefficient between n-3 HUFA, essential fatty acids (EFAs) and their respective ratios in rotifers and pigmentation success and eye migration in yellowtail flounder (*Limanda ferruginea*), n = 9.

EFA and Ratio	Correlation coefficient (r)		
	CP	CEM	CP & CEM
	Experiment 1		
n-3 HUFA	–0.12	**0.86**	0.27
DHA	–0.18	**0.81**	0.23
EPA	0.39	**–0.67**	–0.03
AA	0.21	**0.94**	0.54
DHA/EPA	–0.20	**0.80**	0.22
EPA/AA	0.11	**–0.68**	–0.37
DHA/AA	–0.32	**0.78**	0.03
	Experiment 2		
n-3 HUFA	0.65	0.60	0.35
DHA	0.53	0.56	0.26
EPA	–0.09	–0.39	0.02
AA	**0.71**	0.48	0.34
DHA/EPA	0.51	0.53	0.22
EPA/AA	–0.55	–0.46	–0.20
DHA/AA	–0.33	0.27	–0.07

CP, complete pigmentation; CEM, complete eye migration; CP&CEM, complete pigmentation and eye migration. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; HUFA, highly unsaturated fatty acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid. Bold values indicate significance at 0.05 level.

inversely correlated with the eye migration ($r = -0.67$; $p = 0.048$ and $r = -0.68$; $p = 0.046$, respectively) of the fish. In the second experiment, no correlation was found between the n-3 HUFA, EFA contents and EFA ratios and the complete pigmentation, eye migration and complete pigmentation + eye migration ($r = -0.55$ to 0.65; $p = 0.06 - 0.96$) ($r = -0.49$ to 0.54; $p>0.05$), except for AA content, which was found to correlate with the complete pigmentation ($r = 0.71$; $p = 0.03$) of the juvenile flounder.

3.4 Discussion

3.4.1 Survival

The treatments in the present study resulted in variable survival rates of yellowtail flounder larvae (6 – 20%). In each experimental set-up, the survival of fish was significantly higher in larvae provided live prey enriched with seal oil emulsion and was significantly lower in fish fed Algamac-enriched live prey. The survival of flounder larvae was probably not limited by the fatty acid composition of the live feeds used as evident from the higher survival in larvae fed seal oil. The fatty acid composition, as well as the essential fatty acid contents and their respective ratios, of Algamac-enriched live prey was clearly better compared to that of live prey enriched with other oil emulsions and should favour larval fish requirements, yet it resulted in lower survival. Despite the much lower DHA content in live feeds used in this experiment, the survival of yellowtail flounder larvae were, however, comparable to those of yellowtail flounder larvae reported by Copeman *et al.* (2002). It must be pointed out that the occurrence of the pink-coloured microorganisms in tanks under Algamac feeding was more prevalent

than in tanks under the other feeding regimes, resulting in much higher mortality, thus masking the effect of not only high DHA and DHA/EPA but also n-3 HUFA, AA and DHA/AA ratio on survival of the larvae.

Rainuzzo *et al.* (1997) suggested that increased n-3 HUFA content in live feed is probably not the only factor that enhances the survival and growth of fish larvae, but the DHA/EPA ratio and their individual contents in absolute terms are also important for larval fish nutrition. In fact, the specific role of DHA and EPA during larval development are different (Watanabe, 1993). Lisac *et al.* (1986) reported good correlation of larval survival with EPA, but DHA improved larval quality and growth. Takeuchi *et al.* (1998) reported that both survival and vitality of larvae during *Artemia* feeding stages were affected by the DHA levels in rotifers at the start of feeding. These authors found that as the DHA content in rotifers increased, the requirement of larvae for this fatty acid during *Artemia* feeding stages decreased from 2.6 to 1.6% (dw). The ratio of DHA to EPA also significantly affects the survival of marine fish larvae. Increasing the DHA/EPA ratio from 0.1 to 0.5 in the diet of turbot juveniles markedly increased survival (Bell *et al.,* 1985). The yolks of marine fish eggs contain a DHA/EPA ratio of about 2 (Parrish *et al.*, 1994; Sargent, 1995), which further suggests a requirement for a high DHA/EPA ratio in the first feeding larvae.

In both experiments, the occurrence of pink-coloured microorganisms was prevalent from day-4 of feeding, coinciding with the occurrence of high rates of mortality (day-3 to 10 of feeding). High rates of mortality, however, again took place when co-feeding rotifers with *Artemia* was started (day-27 to 30 of feeding experiments). This second peak of mortality might have been due to different bacterial communities

associated with *Artemia.* In the first experiment, the occurrence of pink-coloured microorganisms was noticeable every other day after rearing tanks were cleaned, particularly in Algamac feeding tanks, while in the second experiment it was persistently noticeable on a daily basis (about 16 h after tanks were cleaned). These microorganisms consisted mainly of cyanobacteria (probably *Entophysalis*) and filamentous red algae, probably the *Trailiella* phase of *Bonnemaisonia hamifera* (Dr. Alan Whittick, personal communication). It was observed that larvae having these pink-coloured microorganisms in their gut all died within a day. This was coupled with the fact that there was a high proportion of "gape" larvae in the population (28 and 36% for the first and the second experiment, respectively). Gape larvae are characterized by their mouths covered by a thin membrane (buccal septum) and/or a malformed lower jaw making them unable to feed. These "gaped" larvae eventually died when their endogenous food reserve was completely depleted.

3.4.2 Growth

The growth of flounder larvae decreased continuously throughout the experimental period. In each experimental set-up, the size and the specific growth rates were similar among feeding treatments for each sampling date, indicating that the gross energy requirements of the flounder larvae/fry were met approximately equally by each of the feeding regimes. Fish fed Algamac-enriched live prey in the first experiment showed a marginally higher growth rate compared to the growth rates of larvae fed seal oil and menhaden oil-enriched diets. Whether this marginally higher growth rate was due to the much higher contents of DHA and AA in Algamac diets, or by some other factors,

is not known. In the second experiment, higher DHA content did not exert any effect on the growth of the fish. It is worth mentioning that the absence of growth promoting effect of DHA might have been due to the fact that larger larvae, as shown in Fig. 3.1, died during the occurrence of high mortality as a result of high intensity of pink-coloured microorganism infestation. Although DHA has been reported to promote better growth in many fish species, such as turbot (Gatesoupe and Le Millinaire, 1985), Japanese flounder and red seabream (Izquierdo *et al.*, 1989), gilthead seabream (Koven *et al.*, 1993; Rainuzzo *et al.*, 1997) and yellowtail flounder (Copeman *et al.*, 2002), it has also been reported that the relative effects of DHA and EPA as growth enhancers vary somewhat with species (Takeuchi and Watanabe, 1982; Castell *et al.*, 1994; Koven *et al.*, 2000).

The growth of yellowtail flounder larvae in this experiment were comparable to those reported by Copeman *et al.* (2002; 7.3 – 9.7 mm) and Rabe and Brown (2000; 9.0 mm). The former authors used rotifers enriched with specially formulated emulsions containing high levels of EFA, whereas the latter used rotifers that were maintained on culture Selco and enriched with microalgae *Isochrysis galbana* (T-Iso) using a pulse feeding strategy. In the present experiment, larval flounder reached a total length of 8.1 mm after three weeks and 16.9 mm after five weeks of feeding. These values were comparable with the studies mentioned above considering that the length of the caudal (tail) fin of larvae at 4 to 5 weeks after hatch is usually in the range of 2 to 3 mm. It is not possible to compare the specific growth rates of larvae from this experiment to the findings of Copeman *et al.*, (2002) and Rabe and Brown (2000) since they did not use specific growth rate to express the growth of their experimental larvae.

It should be emphasized that since experimental fish were fed only twice daily, the EFA intake of larvae might have been insufficient to meet the amounts required to promote better growth. This was coupled with a long residence time of *Artemia* in the experimental tanks. It is well documented that live prey, especially *Artemia*, catabolize EFA rapidly, particularly DHA, during starvation. When subjecting the 24-h enriched nauplii to a 24-h starvation, fatty acids, including HUFA, are mobilized from reserve neural lipids and used as a substrate for energy production (Han *et al.*, 2001). The rapid catabolism of DHA during starvation is a well-known feature in most *Artemia* species (Danielsen *et al.*, 1995; Evjemo *et al.*, 1997; Estévez *et al.*, 1998) and has been contrasted to the simultaneous observations of a preferential conservation of EPA (Han *et al.*, 2001). In this experiment, although the flow rate of water into the tanks was sufficient to achieve a complete water turn-over in 2 to 3 h, the number of *Artemia* remaining in the tanks 15 to 16 h after feeding was still significant (up to 0.4 prey/mL). The DHA content in *Artemia* collected prior to the morning feeding (left-over) had decreased by 88, 75 and 24% in seal oil, menhaden oil and Algamac treatment, respectively. It appears that lower content of DHA in live prey (2.65, 3.50 of total fatty acids in seal oil and menhaden oil diets, respectively) results in a much higher catabolism rate of this fatty acid during starvation compared to when diets contain a higher DHA level (15.2% in Algamac diet). Similarly, the content of AA had decreased dramatically in *Artemia* previously enriched with seal oil and menhaden oil, but surprisingly, its proportion (% fatty acids) increased in starving Algamac enriched *Artemia*, although its proportion (% dry wt.) remained similar to that of non-starved one. On the other hand, the proportion of EPA slightly

increased in all starved *Artemia*, indicating accumulation of some chain shortening products of DHA.

The importance of n-3 HUFA for marine fish larvae has been widely studied and their requirements have been reported for a number of species. Among marine species, the n-3 HUFA requirement of larvae varies considerably, and has been reported to be above 3.9% for Japanese yellowtail, 3.5% for red sea bream, 1.8 - 3.5% for Japanese flounder, 1.2 - 3.2% for turbot, 1.0% for sea bass and 0.9% for sole (Izquierdo, 1996). In the present experiments, the n-3 HUFA content in rotifers was between 3.15 and 4.90% (dry wt.) in the first experiment and 3.15 and 5.52% in the second experiment. In *Artemia,* the n-3 HUFA content ranged from 1.79 to 5.61% and from 1.55 to 3.47% (dry wt.) in the first and second experiment, respectively. Despite the presence of different n-3 HUFA content in diets, the growth rates of larval yellowtail flounder were essentially similar among treatments for each experimental set-up. For yellowtail flounder, it is likely that the n-3 HUFA requirement for this species is about 3.5% (dry wt.) in rotifers and 2% in *Artemia*, similar to those for Japanese flounder and turbot. Further research is, however, needed to verify this assumption. These values can also be influenced by dietary factors, for instance the n-3 PUFA requirement is positively correlated with the total dietary lipid level (Howel *et al.*, 1998).

3.4.3 Pigmentation

Malpigmentation of flatfish is characterized by either a deficiency of pigment cells on portions of the ocular side (albinism, pseudoalbinism or hypomelanism) or excess pigmentation on the blind side (staining, spotting or ambicolouration). Diet related

differences on pigmentation of flatfish has been studied through a number of nutritional experiments. Yamamoto *et al.* (1992) fed Japanese flounder *Paralichthys olivaceus* either rotifers and/or *Artemia*, or enriched rotifers and a formulated larval diet or red sea bream (*Pagrus major*) eggs, and found that fish given red sea bream eggs exhibited the lowest occurrence of malpigmentation. Other studies reported a reduction in malpigmentation of over 20% by feeding the larvae with wild zooplankton (Nakanishi and Fujita, 1986; Seikai, 1989). Seikai (1989) claimed that 100% normally pigmented juvenile flounder can be produced using wild zooplankton as larval food and almost 100% total albinism by feeding the larvae Brazilian *Artemia* nauplii and rotifers.

In the present study, normal pigmentation rates of yellowtail flounder in the present experiment ranged from 43 to 53% of the fish population. These values were similar with those (39 – 47%) for yellowtail flounder reported previously by Copeman *et al.* (2002), despite the fact that diets used by these authors contained EFA levels much higher compared to the EFA levels in seal oil, menhaden oil and seal oil+DHASCO diets, but similar to those levels in Algamac-fed rotifers used in the present study. Depending upon feeding regime, up to 45% of yellowtail flounder juveniles exhibited 'perfect' metamorphic characteristics, i.e., correct distribution of pigment and complete migration of the eyes to the ocular side. The proportion of perfect juveniles in the first experiment was highest in fish fed menhaden oil-enriched live prey (45%) and lowest in fish fed seal oil-enriched live prey (31%). While no significant difference was found in the proportion of juvenile that underwent complete pigmentation and eye migration between menhaden oil and Algamac (41%) feeding treatments, both treatments were higher ($p<0.05$) than that of seal oil treatment. In the second experiment, however, this parameter did not

differ among treatments (26, 25 and 28% for seal oil, seal oil+DHASCO and Algamac feeding, respectively).

The present experiments with yellowtail flounder showed no relationship between the rate of normal pigmentation and the n-3 HUFA, EFA contents and EFA ratios in live feeds, except in one case where AA content of rotifers positively and significantly correlated with the normal pigmentation. Previously, Copeman *et al.* (2002) reported that AA negatively correlated with normal pigmentation. However, a high malpigmentation in their study occurred only in fish that received diets containing a high level (7% of total fatty acids) AA. In fact, their experimental fish receiving AA at levels between 0.7 – 2.2% exhibited normal pigmentation rates similar to the result from the present study (AA level was 0.54 – 2.29% of total fatty acids). From these two studies, it can be concluded the dietary AA requirement of yellowtail flounder is 1 – 2 % of the total fatty acids. Further research is, however, needed to clarify this conclusion.

A DHA/EPA ratio of 2 has been suggested as an adequate dietary level for marine fish larvae (Sargent *et al.*, 1999). However, Copeman *et al.* (2002) indicated that ratios above 3 are required for yellowtail flounder in order to achieve high rates of normal pigmentation. In the present study, a DHA/EPA ratio of 1 – 1.2 was sufficient to achieve a normal pigmentation rate of up to 53%, which was higher than the highest normal pigmentation rate (47%) reported by Copeman *et al.* (2002). It is, therefore, logical to propose that the DHA/EPA ratio required by the yellowtail flounder larvae in their diets is around 1.2. Considering the overall discrepancies from these two studies on yellowtail flounder, it is clear dietary EFA should not only present in the proper amounts, but also in proper ratios, as has been suggested for larval marine fish by Sargent *et al.* (1999).

Dietary imbalances in HUFA during larval development may affect growth, survival and pigmentation of fish larvae (Reitan *et al.,* 1994; Estévez *et al.,* 1999; Sargent *et al.,* 1999).

There are some possible explanations underlying the absence of significant differences in the rates of pigmentation in the present experiment. First, the daily intake of DHA by larvae was too low or sub-optimal to meet the larval flounder requirement to promote good pigmentation due to a low feeding frequency. Second, live prey used may have been deficient in other essential nutrient, such as vitamins A and D, zinc, iron and riboflavin, among others. These nutrients have been reported to play an important role in the success of flatfish pigmentation. Nakamura *et al.* (1986) also ascribed the insufficient production of melanin to a deficiency of photosensitive substances, such as riboflavin, carotenoids and vitamins A and D. Rønnestad *et al.* (1998a) found that halibut larvae fed *Artemia* contained 50 to 80% less vitamin A compared to larvae fed copepods. Vitamin A is critical for a whole range of functions in larval fish, including pigmentation, stress tolerance and formation of the retinal opsins needed for larvae to begin visual feeding (Dhert *et al.,* 1994; Rønnestad *et al.,* 1998b).

Environmental conditions, such as rearing substrate and light may have also been contributing factors in malpigmentation of flatfish. It has been reported that culture conditions after metamorphosis also affect the abnormality on the blind side (Iwata *et al.*, 1995). The colour abnormality may be prevented by the presence of sand, such as microceramic sand, on the bottom where the larvae undergo metamorphosis. Blind-side pigmentation developed gradually in Atlantic halibut juveniles held in smooth-bottomed tanks (Ottesen and Strand, 1996). Seikai (1991) reported that exposure to fluorescent

light can cause the staining form of hypermelanosis in Japanese flounder. Iwata and Kikuchi (1998) found that the inability to bury is significantly more important than light exposure in causing blind-side hypermelanosis in Japanese flounder. Considering the results of the present study and those of Copeman *et al.* (2002), it appears that, in yellowtail flounder, in addition to dietary factors, rearing environment may also play an important role in the determination of pigmentation success. The latter, however, requires further studies.

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Chapter 4

Fatty Acid Composition of Total Lipids of Yellowtail Flounder (*Limanda ferruginea*) Fed Live Feeds Enriched with Various Oil Emulsions

4.1 Introduction

Body lipid in cultured fish often reflects the dietary intake. Many studies have been carried out on the effect of dietary fatty acid composition on fatty acid metabolism, especially in connection with the essential fatty acid requirements of fish species, and there is no doubt that the fatty acid composition of fish reflects, to a large extent, that of the diet (Kennish *et al.,* 1992; Dos Santos *et al.,* 1993; Fair *et al.,* 1993; Rodriguez *et al.,* 1994; Guillou *et al.,* 1995; Eatevez *et al.,* 1997). For example, Kennish *et al.* (1992) reported that rearing salmon on different diets caused changes in lipid content and classes, PUFA, n-3 and n-6 fatty acids, and that their changes reflected those in the diets.

In general, fish fed on diets containing high lipid content also accumulate high lipid levels in their tissues. Dos Santos *et al.* (1993) observed, however, that neither the dietary type nor the feeding regime had any marked influence upon the total lipid content of muscle tissue of cod, a white lean fish. These authors further reported that the type of diet supplied appeared to influence both the relative proportions of lipid classes and the fatty acid composition of the muscle tissues. By exerting control over the dietary nutrients of cultured fish, aquaculturists can raise fish with consistent proportions of desirable fatty acids. Changes in specific fatty acid profiles may occur depending on the

type of oil fed or other factors such as fish size, total dietary lipid or the length of the feeding period (Fair *et al.,* 1993). However, an increase in the quantity of tissue lipid does not necessarily reflect increases in favourable fatty acids.

As early as thirty years ago, investigations conducted by Castell *et al.* (1972) showed that dietary 18:2n-6 raised the concentration of this fatty acid and that of 20:4n-6 and 22:5n-6, while dietary 18:3n-3 increased the level of this fatty acid and that of 22:6n-3 in the phospholipids and neutral lipids of the body of rainbow trout fingerlings. Boggio *et al.* (1985) showed that administration of feed containing higher levels of n-3 fatty acids (e.g., by adding herring oil) markedly increased the proportion of these fatty acids in the muscle tissue of rainbow trout, compared with controls given a diet containing lard. Similarly, Rodriguez *et al.* (1994) reported that the n-3 HUFA levels in gilthead seabream (*Sparus aurata*) larvae increased proportionally to the content of these fatty acids in rotifers. Fish larvae receiving a diet deficient in n-3 HUFA showed a decrease in their EPA, DPA (22:5n-3) and DHA contents. Guillou *et al.* (1995) found that the accumulation of certain fatty acids in the muscle tissues of brook charr (*Salvelinus fontinalis*) was influenced primarily by their concentration in the diet. However, even though there were important differences in the proportion of saturated and polyunsaturated fatty acids in the experimental diets, fish maintained relatively similar proportions of these fatty acids in their muscle (Guillou *et al.,* 1995). Rodriguez *et al.* (1994) also found that the amounts of 18:1n-9 in fish larvae did not increase in spite of having received a high level of this fatty acid in the diet.

The requirement for C18 PUFA is associated with the ability of fish to convert these fatty acids by elongation and desaturation to the longer chain C20 and C22 PUFAs. Longer chain PUFA, however, especially the n-3 series, may also exert feedback inhibition on the desaturation of C18 PUFAs (Olsen *et al.,* 1990). Olsen and Ringo (1992) showed that feeding a PUFA-supplemented diet to fish virtually diminished elongation and desaturation in both polar and neutral lipid classes, showing the importance of diet on lipogenic processes. Similarly, Buzzi *et al.* (1996) reported that the presence of fish oil containing preformed EPA and DHA in the diet of trout resulted in a marked decrease in the rates of formation of DHA from 18:3n-3.

The ability of long chain n-3 PUFAs to regulate the transcription of genes coding for lipogenic enzymes is well acknowledged (Clarke and Jump, 1994) and a recent study has provided evidence that the reduction of Δ9 desaturase mRNA production in rat liver due to dietary fish oil may be specifically attributable to DHA (Lochsen *et al.,* 1997). Henderson *et al.* (1998) investigated the effects of DHA, tetracosapentaenoic (24:5n-3) and tetracosahexaenoic (24:6n-3) acids and concluded that in the conversion of 18:3n-3 to 24:6n-3 by trout liver microsomes, the Δ6 desaturation of 18:3n-3 may be subjected to direct feedback inhibition and that 24:5n-3 may be preferred over 18:3n-3 as a substrate for Δ6 desaturation. It is possible that PUFAs can also exert post-transcriptional influences on desaturase activity by changing the fatty acid composition of the phospholipids in the membranes surrounding the enzymes, thus regulating the enzyme's activity (Giron *et al.,* 1996). Marine fish seem to lack one or more of the desaturation enzymes necessary to accomplish this process, and strictly speaking, therefore, only

DHA, EPA and AA can be termed essential fatty acids in these fish (Sargent *et al.,* 1989). Thus, the objective of this study was to determine the effects of various oil emulsions on the fatty acid profiles of live feeds and their subsequent effects on the fatty acid composition of larval and juvenile yellowtail flounder (*Limanda ferruginea*).

4.2 Materials and methods

Refer to Chapter 2, section 2.1.6, sub-section 2.1.6.1 to 2.1.6.3.

4.3 Results

4.3.1 Live feeds

The total lipid contents of enriched live feeds used during studies on yellowtail flounder are presented in Table 4.1. As expected, rotifers and *Artemia* enriched with seal oil, menhaden oil and seal oil+DHASCO contained higher ($p<0.05$) lipid content compared to Algamac-enriched live feeds due to the lower lipid content in the latter enrichment medium. The total lipid contents of rotifers or *Artemia* enriched with the same oil emulsions (seal oil or Algamac) were similar between the first and the second experiments.

Fatty acid composition of rotifers and *Artemia* enriched with different oil emulsions is given in Appendices 3.1 – 3.4. Rotifers enriched with seal oil, menhaden oil and seal oil+DHASCO contained monounsaturated fatty acids (MUFAs) as their dominant fatty acids, followed by polyunsaturated fatty acids (PUFAs) and saturated fatty acids (SFAs) (Chapter 3, Table 3.2 and 3.3). On the other hand, rotifers enriched with

Table 4.1. Total lipid content (% dry wt.) of live prey used during yellowtail flounder (*Limanda ferruginea*) experiments.

Emulsion	Rotifers	*Artemia*
First experiment:		
Original	16.4 ± 0.26^{a} (3.02)	14.9 ± 0.37^{a} (2.88)
Seal oil	20.2 ± 0.34^{b} (3.43)	19.9 ± 0.64^{b} (3.68)
Menhaden oil	19.9 ± 0.61^{b} (3.35)	18.7 ± 0.49^{b} (3.34)
Algamac-3010	18.5 ± 0.42^{c} (3.27)	15.5 ± 1.03^{a} (3.22)
Second experiment:		
Original	17.0 ± 0.42^{a} (3.12)	15.3 ± 0.44^{a} (2.97)
Seal oil	19.2 ± 0.65^{b} (3.38)	20.9 ± 0.57^{b} (3.59)
Seal oil + DHASCO	20.2 ± 0.38^{b} (3.36)	18.8 ± 0.88^{c} (3.41)
Algamac-3010	17.9 ± 0.81^{a} (3.30)	16.2 ± 0.76^{a} (3.14)

Values are means ± standard deviations of 5 replicates. DHASCO, DHA-rich single cell oil.
Values in each column under each experimental set-up with different superscripts (a,b,c) are different ($p<0.05$) from one another.
Values in brackets represent lipid content (%) on a wet weight basis.

Algamac contained PUFAs as their dominant fatty acids, followed by MUFAs and SFAs in the first experiment, and followed by SFAs and MUFAs in the second experiment. The proportion of n-3 HUFAs in Algamac-enriched rotifers was higher ($p<0.05$) than those of seal oil-, menhaden oil- and seal oil+DHASCO-enriched rotifers. While the proportions of DHA and AA were higher ($p<0.05$) in Algamac-enriched rotifers, the proportion of EPA was lower ($p<0.05$) than those of rotifers enriched with seal oil, menhaden oil and seal oil+DHASCO. The ratio of DHA to EPA ranged from 1.18 to 8.02, being highest in the Algamac- and lowest in menhaden oil-fed rotifers. It is interesting that even though the proportion of AA in Algamac was similar to that of seal oil and much lower than that of menhaden oil, yet it resulted in a higher ($p<0.05$) proportion in Algamac-enriched rotifers.

In *Artemia*, PUFAs constituted the major fatty acids followed by MUFAs and SFAs, except in seal oil-enriched *Artemia* used in the second experiment where MUFAs exhibited the highest proportion (Chapter 3, Table 3.2 and 3.3). As in rotifers, *Artemia* enriched with Algamac also contained a higher ($p<0.05$) proportion of n-3 HUFAs compared to the other treatments. While DHA and AA contents were higher ($p<0.05$) in Algamac-enriched *Artemia*, the contents of EPA showed only marginal, but significant ($p<0.05$), differences among treatments for each experimental set-up. Similar to that of rotifers, the DHA/EPA ratio in Algamac-enriched *Artemia* was higher ($p<0.05$) than that of other treatments. This ratio ranged from 0.35 in seal oil- to 2.91 in Algamac-enriched *Artemia*.

In uneaten *Artemia*, MUFAs constituted the highest percentage of fatty acids of seal oil-enriched prey followed by PUFAs and SFAs. In menhaden oil and Algamac treatments, the fatty acids were dominated by PUFAs followed by MUFAs and SFAs (Chapter 3, Table 3.4). The proportion of n-3 HUFAs decreased from 15, 14 and 30% in newly enriched *Artemia* with seal oil, menhaden oil and Algamac, respectively, to 10, 11 and 27% in the left-over prey. While the proportion of DHA in all treatments and AA in seal oil and menhaden oil treatment decreased, the proportion of EPA in all treatments and AA in Algamac treatment increased. Similarly, the DHA/EPA and DHA/AA ratios decreased drastically in all treatments. On the other hand, the EPA/AA ratio in seal oil and menhaden oil treatment increased drastically, whereas that of Algamac treatment remained relatively unchanged.

4.3.2 One-day post first feeding yellowtail flounder larvae

The fatty acid composition of total lipids of yellowtail flounder larvae used in this experiment is given in Table 4.2. One-day post first feeding larvae used in the first and second experiments displayed similar fatty acid composition of their total lipids. The proportion of each fatty acid, however, showed some differences ($p<0.05$) between the two larval flounder populations. The dominant fatty acids were DHA, palmitic (16:0) and oleic (18:1n-9) acids. Essential fatty acids (EFAs), DHA, EPA and AA, constituted 18.9, 4.76 and 1.48%, respectively, of the total fatty acids of larvae in the first experiment, and 17.4, 5.33 and 1.62%, respectively, in the second experiment. Of the EFA contents, only that of AA was found to be different ($p<0.05$) between larvae used in the first and second experiments. The DHA/EPA ratio was 3.98 for larvae in the first

Table 4.2. Fatty acid composition (%) of one day post first feeding yellowtail flounder (*Limanda ferruginea*) larvae used in feeding experiment with enriched live prey.

Fatty acid	First experiment		Second experiment	
	Mean	SD	Mean	SD
12:0	0.31[a]	0.03	0.25[b]	0.01
14:0	3.59[a]	0.31	2.65[b]	0.13
14:1n-5	0.75[a]	0.06	0.61[b]	0.05
15:0	1.72[a]	0.12	0.75[b]	0.02
16:0	17.9[a]	1.52	18.6[a]	0.80
16:1n-7	4.20[a]	0.36	4.25[a]	0.22
16:2n-4	2.94[a]	0.25	2.40[b]	0.19
16:3n-4	1.59[a]	0.14	1.38[a]	0.10
16:4n-3	0.58[a]	0.04	-	-
17:0	0.77[a]	0.06	0.99[b]	0.01
17:1	0.73[a]	0.06	0.58[b]	0.07
18:0	5.79[a]	0.49	7.82[b]	0.26
18:1n-11	2.69[a]	0.23	1.86[b]	0.14
18:1n-9	11.3[a]	0.95	8.39[b]	0.38
18:1n-7	2.01[a]	0.17	1.65[b]	0.13
18:2n-6	3.39[a]	0.29	3.67[a]	0.15
18:3n-6	1.12[a]	0.09	1.33[b]	0.04
18:3n-3	2.22[a]	0.18	2.73[b]	0.14
18:4n-6	1.45[a]	0.12	0.46[b]	0.03
18:4n-3	1.87[a]	0.12	1.52[b]	0.08
20:0	0.79[a]	0.05	0.54[b]	0.01
20:1n-11	0.47[a]	0.03	0.34[b]	0.04
20:1n-9	2.02[a]	0.17	2.12[a]	0.12
20:4n-6	1.48[a]	0.12	1.62[b]	0.06
20:5n-3	4.76[a]	0.40	5.33[a]	0.15
22:2n-6	-	-	0.50[b]	0.01
22:4n-3	1.29[a]	0.11	2.23[b]	0.04
22:5n-3	1.77[a]	0.14	0.56[b]	0.04
22:6n-3	18.9[a]	1.61	17.4[a]	1.20
24:1	-	-	1.46[b]	0.04
DHA/EPA	3.98[a]	0.14	3.28[b]	0.32
EPA/AA	3.21[a]	0.13	3.29[a]	0.21
DHA/AA	12.8[a]	0.39	10.7[b]	0.34

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. Values in each row with different superscripts (a,b) are different ($p<0.05$). DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

experiment and 3.28 in the second experiment. Significant difference in EFA ratios was not shown only by that of EPA/AA.

One day post first feeding larvae used in the first experiment contained 31% SFAs, while that of MUFAs and PUFAs were present at 24 and 43% of the total fatty acids, respectively. The n-3 HUFA accounted for 29% and n-6 fatty acids constituted up to 7.4% of the total fatty acids. Lipids of the larvae employed in the second experiment contained 31% SFAs, 21% MUFAs and 41% PUFAs; n-3 HUFA comprised 27% and n-6 fatty acids accounted for 7.1% of the total fatty acids.

4.3.3 Twenty-day post first feeding yellowtail flounder larvae

The fatty acid composition of total lipids of twenty-day post first feeding larvae fed rotifers enriched with seal oil, menhaden oil and Algamac is given in Table 4.3. Larval flounder under different feeding treatments displayed similar fatty acids, which were dominant in their lipids. These fatty acids were 16:0, 18:1n-9 and 22:6n-3. The most abundant saturated fatty acid in flounder larvae for all treatments was 16:0. This was followed by stearic acid (18:0) in seal oil treatment, and by 18:0 and 14:0, which existed at a similar level in both the menhaden and Algamac treatments. Palmitoleic acid (16:1n-7) was the second most dominant MUFAs present in all treatments.

The EFAs of flounder larvae reflected those of their rotifer diets (Fig. 4.1). Larval fish under seal oil and menhaden oil feeding regimes showed similar ($p>0.05$) DHA, EPA and AA contents, whereas those of larvae under Algamac treatment were different ($p<0.05$) from the other two treatments. In Algamac feeding, both the DHA (14.8%) and AA (3.20%) contents were higher ($p<0.05$) compared to those in seal oil

Table 4.3. Fatty acid composition (%) of total lipid of yellowtail flounder (*Limanda ferruginea*) at 20-days post first feeding reared on rotifers (*Brachionus plicatilis*) enriched with different oil emulsions (first experiment).

Fatty acid	Yellowtail larvae fed on rotifers enriched with:					
	Seal oil		Menhaden oil		Algamac-3010	
	Mean	SD	Mean	SD	Mean	SD
12:0	0.14^{a}	0.01	0.24^{b}	0.02	0.20^{b}	0.02
14:0	2.58^{a}	0.18	3.25^{b}	0.29	3.21^{b}	0.26
14:1n-5	0.51^{a}	0.03	0.57^{a}	0.04	0.49^{a}	0.04
15:0	0.54^{a}	0.03	0.67^{b}	0.04	0.64^{ab}	0.05
16:0	10.1^{a}	0.72	10.0^{a}	0.88	10.7^{a}	0.86
16:1n-7	8.72^{a}	0.62	7.05^{b}	0.62	5.89^{b}	0.48
16:2n-4	0.49^{a}	0.03	0.41^{b}	0.03	0.32^{c}	0.02
16:3n-4	1.11^{a}	0.08	1.79^{b}	0.15	1.46^{c}	0.12
16:4n-3	0.44^{a}	0.03	0.58^{b}	0.05	0.27^{c}	0.02
17:0	0.40^{a}	0.03	0.30^{b}	0.03	0.40^{a}	0.03
17:1	0.50^{a}	0.04	0.55^{a}	0.04	0.39^{b}	0.03
18:0	4.20^{a}	0.30	3.43^{b}	0.30	3.31^{b}	0.27
18:1n-11	1.24^{a}	0.09	2.25^{b}	0.18	1.92^{b}	0.15
18:1n-9	16.1^{a}	1.15	15.9^{a}	0.87	12.6^{b}	0.79
18:1n-7	4.58^{a}	0.33	4.31^{b}	0.29	3.81^{b}	0.23
18:2n-6	4.03^{a}	0.29	4.12^{a}	0.36	3.39^{a}	0.28
18:3n-6	0.32^{a}	0.02	0.46^{b}	0.04	0.67^{c}	0.05
18:3n-3	3.60^{a}	0.26	3.66^{a}	0.32	2.69^{b}	0.22
18:4n-6	0.81^{a}	0.05	1.45^{b}	0.12	0.89^{a}	0.07
18:4n-3	0.91^{a}	0.06	1.66^{b}	0.14	2.19^{c}	0.16
20:0	0.51^{a}	0.04	1.00^{b}	0.07	1.08^{b}	0.07
20:1n-11	0.50^{a}	0.03	0.40^{b}	0.03	0.44^{ab}	0.03
20:1n-9	2.53^{a}	0.18	1.21^{b}	0.11	1.38^{b}	0.11
20:2n-6	0.39^{a}	0.02	0.34^{a}	0.02	0.50^{b}	0.03
20:4n-6	2.02^{a}	0.14	2.09^{a}	0.18	3.20^{b}	0.24
20:4n-3	1.04^{a}	0.07	1.75^{b}	0.15	1.00^{a}	0.07
20:5n-3	6.61^{a}	0.47	7.13^{a}	0.63	3.84^{b}	0.30
22:1n-13	0.16^{a}	0.01	0.20^{b}	0.01	-	-
22:1n-11	0.46^{a}	0.03	0.77^{b}	0.04	-	-
22:2n-6	0.22^{a}	0.01	0.34^{b}	0.02	0.35^{b}	0.02
22:4n-6	0.12^{a}	0.01	0.14^{a}	0.01	0.35^{b}	0.02
22:4n-3	1.02^{a}	0.07	1.19^{a}	0.11	3.74^{b}	0.29
22:5n-6	-	-	-	-	1.90^{b}	0.14
22:5n-3	3.72^{a}	0.26	1.83^{b}	0.16	1.99^{b}	0.15
22:6n-3	10.5^{a}	0.85	10.1^{a}	0.88	14.6^{b}	1.14
24:1	0.42^{a}	0.02	0.37^{a}	0.03	0.62^{b}	0.04
DHA/EPA	1.58^{a}	0.08	1.41^{a}	0.05	3.84^{b}	0.14
EPA/AA	3.27^{a}	0.16	3.42^{a}	0.10	1.20^{b}	0.06
DHA/AA	5.18^{a}	0.27	4.82^{ab}	0.20	4.61^{b}	0.14

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. SD values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DPFF, day post-first feeding; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

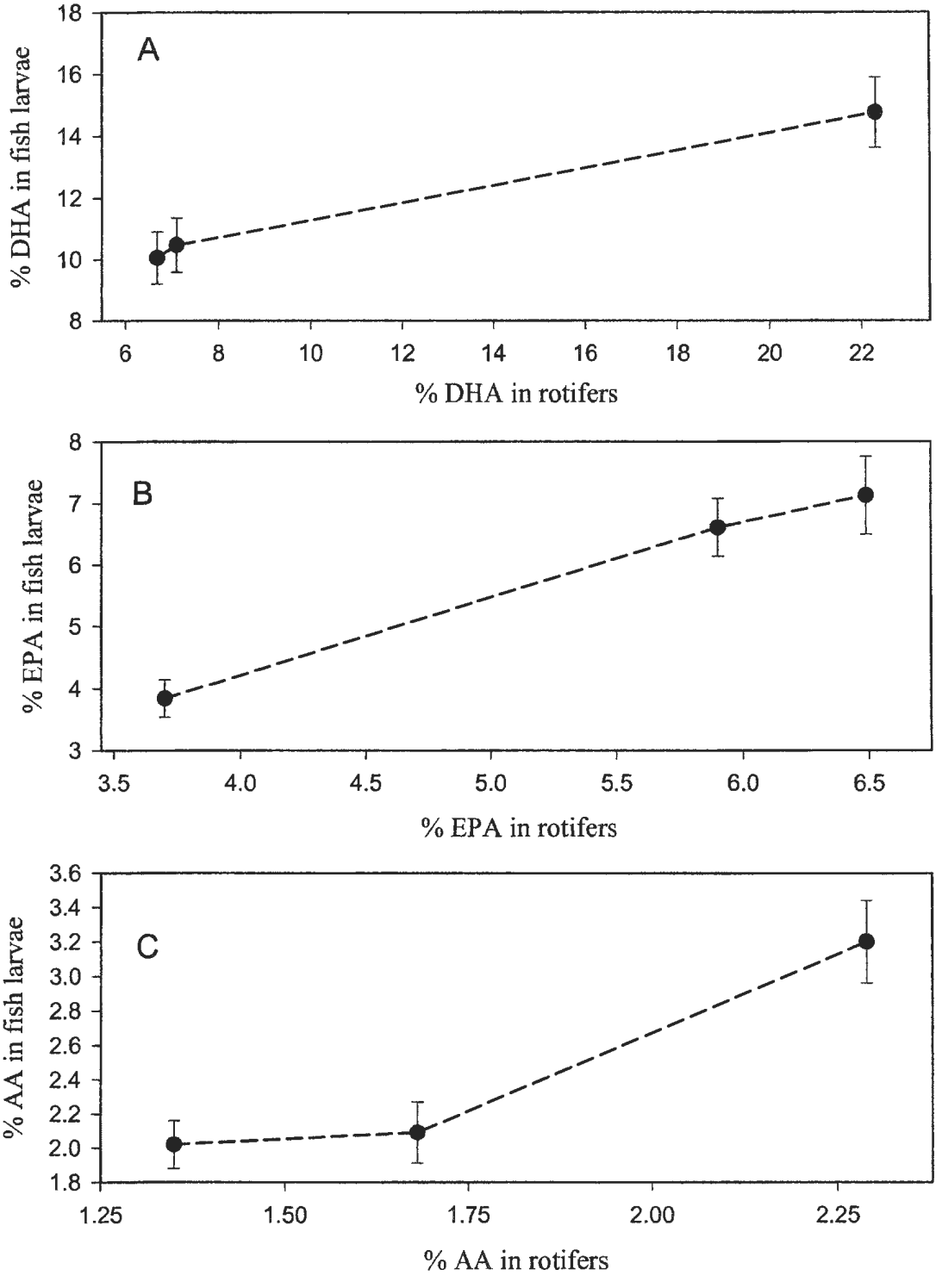

Fig. 4.1. Assimilation of essential fatty acid (EFA) in 20 days post-first feeding (DPFF) yellowtail flounder (*Limanda ferruginea*) larvae fed rotifers enriched with different oil emulsions. Vertical bars represent standard deviations, n = 3. A, B and C - DHA, EPA and AA, respectively.

(10.5 and 2.02%) and menhaden oil (10.1 and 2.09%) treatments, but was lower ($p<0.05$) in its EPA (3.84%) content. Meanwhile, the EPA content was 6.61% and 7.13% in seal oil and menhaden oil treatments, respectively. The DHA/EPA ratio in larvae fed on rotifer enriched with seal and menhaden oil was similar (1.58 and 1.41; $p>0.05$), and was decreased by over 50% compared to the DHA/EPA ratio in the starting larvae (3.98). The DHA/EPA ratio in Algamac treatment was, however, only slightly decreased to 3.84. The DHA content of larvae on day 20 post first feeding under all feeding treatments was significantly lower than the original value (one day post first feeding), whereas the content of EPA and AA increased significantly ($p<0.05$) in the seal oil and menhaden oil treatments. In the Algamac treatment, however, EPA content decreased while AA content increased compared to those in the one day post first feeding larvae.

The fatty acid profile of flounder larvae under each feeding treatment reflected, in general, the fatty acid profile of their respective diets. The level of each fatty acid in larvae also corresponded well to that of the feed although some variation existed (Table 4.3; Appendix 3.1 – 3.4).

4.3.4 Total lipid content of yellowtail flounder juveniles

The lipid content (wet weight) of juvenile flounder ranged from 4.19% in abnormally pigmented fish fed Algamac- to 5.22% in normally pigmented fish fed seal oil-enriched live feeds (Table 4.4). There was no difference ($p>0.05$) in the lipid content between normally and abnormally pigmented fish fed live feeds enriched with the same enrichment medium. However, some differences ($p<0.05$) existed among feeding treatments with seal oil-enriched live feeds resulting in a higher lipid content of fish

Table 4.4. Total lipid content (%, wet wt.) of yellowtail flounder (*Limanda ferruginea*) juveniles fed live feeds enriched with seal oil, menhaden oil, seal oil+DHASCO and Algamac-3010 emulsions.

Feeds enriched with:	Total lipid content (%)			
	Normally pigmented		Abnormally pigmented	
	Mean	SD	Mean	SD
Experiment 1				
Seal oil	5.22^{a}	0.11	5.16^{a}	0.21
Menhaden oil	5.06^{ab}	0.15	4.85^{ab}	0.16
Algamac-3010	4.76^{bc}	0.12	4.63^{b}	0.11
Experiment 2				
Seal oil	4.58^{c}	0.22	4.47^{bc}	0.20
Seal oil + DHASCO	4.55^{c}	0.15	4.66^{b}	0.17
Algamac-3010	4.37^{d}	0.22	4.19^{c}	0.16

Determinations were carried out in triplicates. DHASCO, DHA-rich single cell oil. SD, standard deviation.

Values in each row with different superscripts (x,y) are different ($p<0.05$) from one another.

Values in each column with different superscripts (a,b,c,d) are different ($p<0.05$) from one another.

compared to the other treatments. The trend in the total lipid content of juvenile flounder reflected that of their respective *Artemia* diets (Table 4.1).

4.3.5 Fatty acid composition of normally pigmented yellowtail flounder juveniles

Normally pigmented yellowtail flounder from the first and second experiments displayed similar fatty acid compositions regardless of the feeding treatment (Table 4.5, 4.6 and 4.7). In all feeding regimes, 16:0 and 18:0 were the main SFAs. No differences ($p>0.05$) was observed in these fatty acids among treatments, except in the second experiment where 16:0 content in fish fed Algamac-enriched diet was higher ($p<0.05$) than that of fish fed on a seal oil-enriched diet. The content of 16:0 in fish under seal oil, menhaden oil and Algamac treatments in the first trial was 9.46, 9.63 and 9.80%, respectively, whereas that of 18:0 was 3.58, 3.85 and 3.89%, respectively. In the second experiment, these fatty acids were present at a concentration of 10.1, 10.2 and 10.7% and 4.34, 4.60 and 4.55%, respectively, for flounder under seal oil, seal oil + DHASCO and Algamac feeding regimes. Other saturated fatty acids were present at much lower levels.

The MUFAs in all treatments were dominated by 18:1n-9. The concentration of this acid in the experimental fish ranged from 13.5% in the first experiment to 19.7% in the second experiment. In the first experiment, the content of 18:1n-9 in fish lipid was 15.4, 14.9 and 13.5%, respectively, for seal oil, menhaden oil and Algamac treatments. A much higher content was observed in the second experiment, except for fish under the Algamac feeding where 18:1n-9 showed a similar concentration to that of the first experiment. Fish fed on diets enriched with seal oil and seal oil+DHASCO contained

Table 4.5. Fatty acid composition (%) of total lipids of normally pigmented yellowtail flounder (*Limanda ferruginea*) fed live feeds enriched with seal oil, menhaden oil and Algamac-3010 emulsions for 62 days.

Fatty acid	Yellowtail flounder fed on live feeds enriched with:					
	Seal oil		Menhaden oil		Algamac-3010	
	Mean	SD	Mean	SD	Mean	SD
12:0	0.21^{a}	0.05	0.10^{b}	0.00	-	-
14:0	1.61^{a}	0.11	1.26^{b}	0.09	1.35^{b}	0.03
14:1n-5	0.83^{a}	0.04	0.82^{a}	0.02	0.74^{b}	0.02
15:0	0.46^{a}	0.05	0.32^{b}	0.03	0.32^{b}	0.04
16:0	9.46^{a}	0.65	9.63^{a}	0.37	9.80^{a}	0.67
16:1n-7	6.59^{a}	0.20	4.80^{b}	0.10	3.91^{c}	0.06
16:2n-4	0.50^{a}	0.05	0.58^{b}	0.03	0.46^{a}	0.01
16:3n-4	1.47^{a}	0.09	1.51^{a}	0.13	1.35^{a}	0.17
16:4n-3	0.38^{a}	0.00	0.50^{b}	0.01	0.27^{c}	0.02
17:0	0.44^{a}	0.03	0.54^{b}	0.02	0.64^{c}	0.04
17:1	1.85^{a}	0.13	2.06^{b}	0.07	2.07^{b}	0.06
18:0	3.58^{a}	0.09	3.85^{a}	0.27	3.89^{a}	0.26
18:1n-11	0.71^{a}	0.04	0.68^{a}	0.04	0.83^{b}	0.06
18:1n-9	15.4^{a}	0.64	14.9^{a}	1.02	13.5^{b}	0.47
18:1n-7	5.59^{a}	0.36	5.42^{a}	0.17	5.36^{a}	0.08
18:2n-6	4.38^{a}	0.37	4.49^{a}	0.22	4.15^{a}	0.24
18:3n-6	0.53^{a}	0.03	0.60^{b}	0.04	0.67^{c}	0.03
18:3n-3	17.0^{a}	1.32	18.4^{b}	0.61	18.0^{ab}	0.34
18:4n-6	3.07^{a}	0.10	3.20^{a}	0.22	3.02^{a}	0.05
18:4n-3	0.55^{a}	0.06	0.56^{a}	0.04	0.61^{a}	0.03
20:0	0.55^{a}	0.01	0.44^{b}	0.05	0.31^{c}	0.04
20:1n-11	0.30^{a}	0.01	0.31^{a}	0.01	0.31^{a}	0.02
20:1n-9	1.94^{a}	0.08	0.99^{b}	0.08	0.74^{c}	0.03
20:2n-6	0.18^{a}	0.02	0.23^{b}	0.02	0.24^{b}	0.00
20:4n-6	1.54^{a}	0.08	1.81^{b}	0.05	3.81^{c}	0.15
20:4n-3	1.31^{a}	0.07	0.93^{b}	0.03	0.72^{c}	0.02
20:5n-3	6.11^{a}	0.15	7.19^{b}	0.25	5.84^{c}	0.11
22:0	0.21^{a}	0.01	0.24^{b}	0.02	0.25^{b}	0.01
22:1n-13	0.10^{a}	0.01	0.16^{b}	0.01	0.13^{c}	0.00
22:1n-11	0.26^{a}	0.01	0.10^{b}	0.01	0.12^{c}	0.00
22:2n-6	0.28^{a}	0.01	0.14^{b}	0.01	0.17^{c}	0.01
22:4n-6	0.11^{a}	0.02	0.10^{a}	0.00	0.10^{a}	0.01
22:4n-3	0.45^{a}	0.03	1.01^{b}	0.08	3.13^{c}	0.22
22:5n-3	1.94^{a}	0.10	1.85^{a}	0.17	1.06^{b}	0.08
22:6n-3	5.03^{a}	0.34	5.42^{a}	0.87	11.0^{b}	0.93
DHA/EPA	0.82^{a}	0.04	0.75^{a}	0.05	1.89^{b}	0.05
EPA/AA	3.98^{a}	0.14	3.97^{a}	0.12	1.53^{b}	0.12
DHA/AA	3.28^{a}	0.11	2.99^{b}	0.12	2.90^{b}	0.10

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. SD values < 0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Table 4.6. Fatty acid composition (%) of total lipids of normally pigmented yellowtail flounder (*Limanda ferruginea*) fed live feeds enriched with seal oil, seal oil+DHASCO and Algamac-3010 emulsions for 62 days.

Fatty acid	Yellowtail fed on live prey enriched with:					
	Seal oil		Seal oil + DHASCO		Algamac-3010	
	Mean	SD	Mean	SD	Mean	SD
14:0	1.04[a]	0.04	1.06[a]	0.07	1.25[b]	0.08
14:1n-5	0.50[a]	0.01	0.51[a]	0.03	0.45[b]	0.03
15:0	0.26[a]	0.02	0.23[b]	0.01	0.24[b]	0.01
16:0	10.1[a]	0.23	10.2[ab]	0.48	10.7[b]	0.27
16:1n-7	5.18[a]	0.10	4.79[b]	0.14	2.88[c]	0.08
16:2n-4	0.46	0.01	0.44	0.02	0.47	0.04
16:3n-4	0.82	0.03	0.83	0.02	0.79	0.03
16:4n-3	0.69[a]	0.04	0.38[b]	0.03	0.74[c]	0.01
17:0	0.78[a]	0.04	0.70[b]	0.02	0.67[b]	0.03
17:1	0.98[a]	0.10	0.83[b]	0.09	0.58[c]	0.03
18:0	4.34	0.10	4.60	0.15	4.55	0.32
18:1n-9	19.7[a]	0.38	19.7[a]	1.10	14.7[b]	0.73
18:1n-7	6.39[a]	0. 24	6.02[b]	0.15	5.06[c]	0.18
18:2n-6	5.48[a]	0.17	5.50[a]	0.30	4.13[b]	0.15
18:3n-6	0.68	0.04	0.62	0.03	0.65	0.05
18:3n-3	13.8[a]	0.63	13.4[ab]	0.49	13.0[b]	0.22
18:4n-6	2.61[a]	0.25	2.71[a]	0.11	2.32[b]	0.10
18:4n-3	0.11[a]	0.00	0.12[ab]	0.01	0.13[b]	0.01
20:0	0.37[a]	0.01	0.36[a]	0.03	0.23[b]	0.01
20:1n-11	0.34[a]	0.01	0.33[a]	0.01	0.28[b]	0.02
20:1n-9	2.13[a]	0.15	2.09[a]	0.07	0.98[b]	0.03
20:2n-6	0.31[a]	0.01	0.30[a]	0.01	0.36[b]	0.01
20:4n-6	1.59[a]	0.04	1.54[a]	0.03	3.75[b]	0.11
20:4n-3	1.79[a]	0.02	1.70[b]	0.02	1.74[c]	0.03
20:5n-3	6.68	0.38	6.43	0.29	6.66	0.03
22:0	0.34	0.02	0.33	0.02	0.33	0.05
22:1n-13	-	-	0.15[a]	0.02	-	-
22:1n-11	0.35[a]	0.01	0.30[b]	0.01	0.21[c]	0.02
22:2n-6	0.17[a]	0.00	0.25[b]	0.02	0.70[c]	0.04
22:4n-6	0.28[a]	0.00	1.91[b]	0.14	0.25[a]	0.01
22:4n-3	0.61[a]	0.02	0.74[b]	0.06	0.26[c]	0.02
22:5n-3	1.41[a]	0.03	1.06[b]	0.07	0.98[c]	0.04
22:6n-3	5.13[a]	0.16	6.42[b]	0.37	14.0[c]	0.61
24:1	0.10[a]	0.00	-	-	0.11[a]	0.01
DHA/EPA	0.77[a]	0.03	1.00[b]	0.01	2.10[c]	0.08
EPA/AA	4.21[a]	0.34	4.18[a]	0.12	1.78[b]	0.06
DHA/AA	3.24[a]	0.12	4.17[b]	0.17	3.73[c]	0.27

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. SD values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHASCO, DHA-rich single cell oil; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Table 4.7. The composition of fatty acid groups, EFA and their respective ratios in normally and abnormally pigmented juvenile yellowtail flounder (*Limanda ferruginea*) fed live feeds enriched with different oil emulsions.

Fatty acid	Yellowtail flounder fed live feeds enriched with:					
	Seal oil		Menhaden oil		Algamac-3010	
	NP	AP	NP	AP	NP	AP
First experiment:						
SFAs	16.5	15.5	16.4	14.9	16.6	15.4
MUFAs	33.6	34.4	30.2[a]	34.3[b]	27.7	26.3
PUFAs	44.8[a]	47.1[b]	48.5	48.6	54.6	53.2
n-3-HUFAs	14.5[a]	18.1[b]	17.5	18.2	22.7	20.6
DHA	5.03	5.21	5.42	5.58	11.0	10.9
EPA	6.11	6.23	7.19	7.20	5.84	6.20
AA	1.54	1.71	1.81	1.98	3.81	3.74
DHA/EPA	0.82	0.85	0.75	0.78	1.89	1.76
EPA/AA	3.98	3.65	3.97	3.64	1.53	1.66
DHA/AA	3.28	3.09	2.99	2.82	2.90	2.92
Second experiment:						
	Seal oil		Seal oil +DHASCO		Algamac-3010	
SFAs	17.2	17.3	17.5	18.1	18.0	19.1
MUFAs	35.6	35.4	34.7	34.0	25.2	23.6
PUFAs	42.7	42.8	44.4	43.5	50.9	51.9
n-3-HUFAs	16.4	15.4	16.9	15.6	24.5	26.1
DHA	5.13[a]	3.75[b]	6.42[a]	4.91[b]	14.0[a]	12.2[b]
EPA	6.68	6.61	6.43	6.13	6.66	6.16
AA	1.59	1.63	1.54	1.38	3.75	3.44
DHA/EPA	0.77[a]	0.57[b]	1.00[a]	0.80[b]	2.10	1.98
EPA/AA	4.21	4.07	4.18	4.45	1.78	1.79
DHA/AA	3.24[a]	2.31[b]	4.17[a]	3.58[b]	3.73	3.54

Values are means of triplicate determinations. Typical coefficient of variation is ~5%. NP – normally pigmented; AP – abnormally pigmented.

Values in each row under the same feeding regime with different superscripts (a,b) are different ($p<0.05$) from one another.

SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; HUFAs, highly unsaturated fatty acids; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid; DHASCO, DHA-rich single cell oil.

19.7% of 18:1n-9. Two other MUFAs, 16:1n-7 and 18:1n-7, were also present in considerable amounts (3.91 – 6.59 and 5.06 – 6.39%, respectively).

The major PUFA in all fish was 18:3n-3. The concentration of this fatty acid was higher in the first experiment compared to the second experiment. In the first experiment, 18:3n-3 accounted for 17.0 to 18.4% of the total fatty acids, whereas in the second experiment its concentration was 13.0 to 13.8%, respectively. Although the level of 18:n-3 was maintained in a relatively narrow range in fish, there was a difference ($p<0.05$) in the content of this acid in fish under seal oil and menhaden oil treatments, respectively. A significant ($p<0.05$) difference was also observed in the content of 18:3n-3 between seal oil and Algamac feeding in the second experiment.

Essential fatty acids (DHA and EPA) constituted a significant amount of flounder lipid, particularly in fish fed Algamac-enriched live feeds, while AA was present in a much lower concentration. The DHA content of fish under Algamac feeding was 11.0 and 14.0% in the first and second experiment, respectively, and was higher ($p<0.05$) (twice as much) than those in fish from the other feeding treatments. On the other hand, EPA content in fish fed Algamac-enriched feeds in the first experiment was slightly, but significantly ($p<0.05$), lower than that of fish fed seal oil and menhaden oil-enriched fish (Fig. 4.2A and 4.3A). The EPA content of fish in the second experiment, however, was similar ($p>0.05$) among treatments. Another essential fatty acid, AA, accounted for only 1.54 to 3.81% in fish from the first experiment and 1.54 to 3.75% in the second experiment. Flounder fed seal oil- and menhaden oil-enriched feeds displayed a much lower ($p<0.05$) AA content compared to the fish fed Algamac-enriched feeds. Similarly,

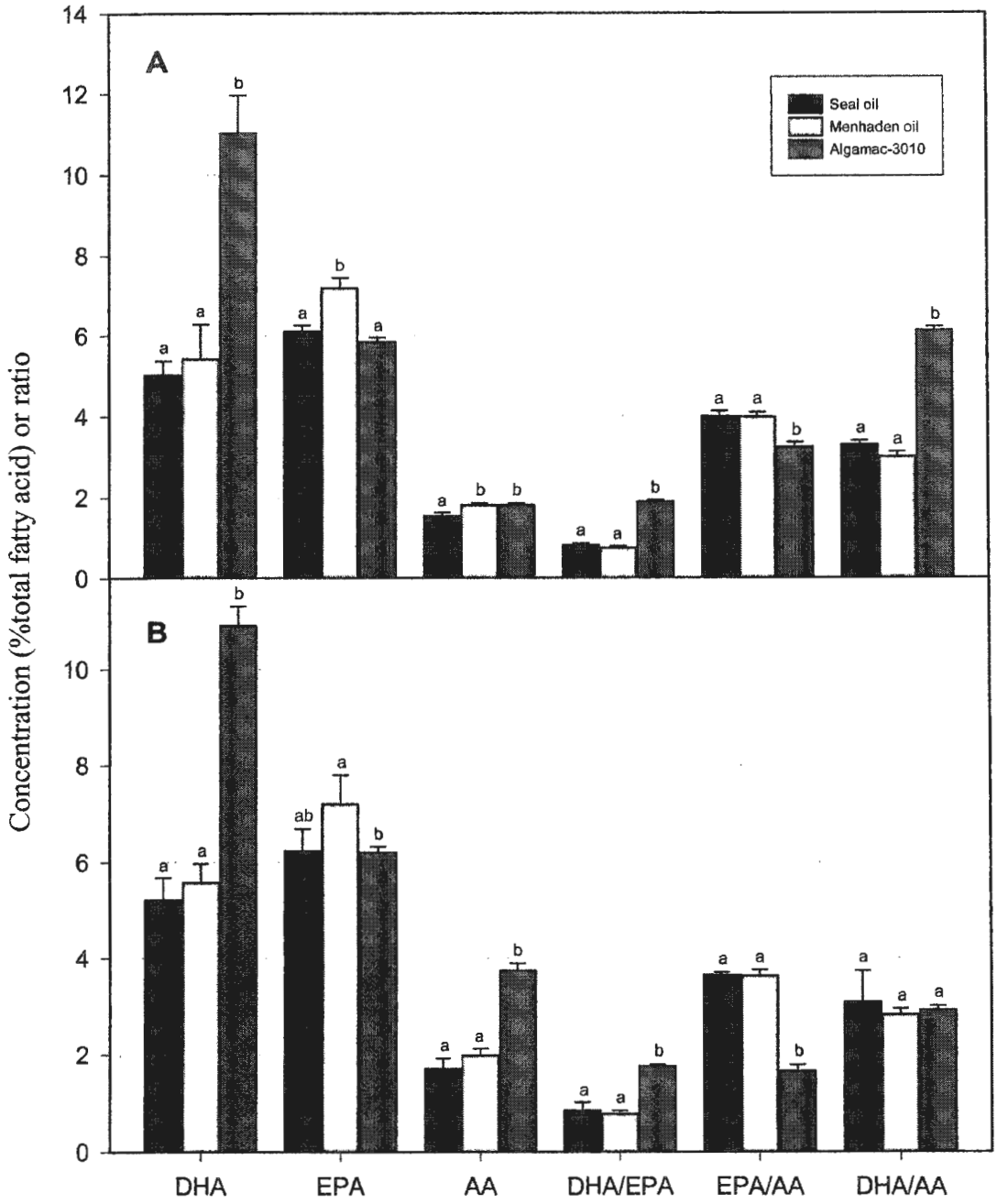

Fig. 4.2. Concentration of DHA, EPA and AA, and their respective ratio in the total lipid of normally (A) and abnormally (B) pigmented yellowtail flounder (*Limanda ferruginea*) fed live feeds enriched with seal oil, menhaden oil and Algamac. Vertical bars represent standard deviation, n = 3. Different letters (a,b,c) indicate significant differences at 0.05 level.

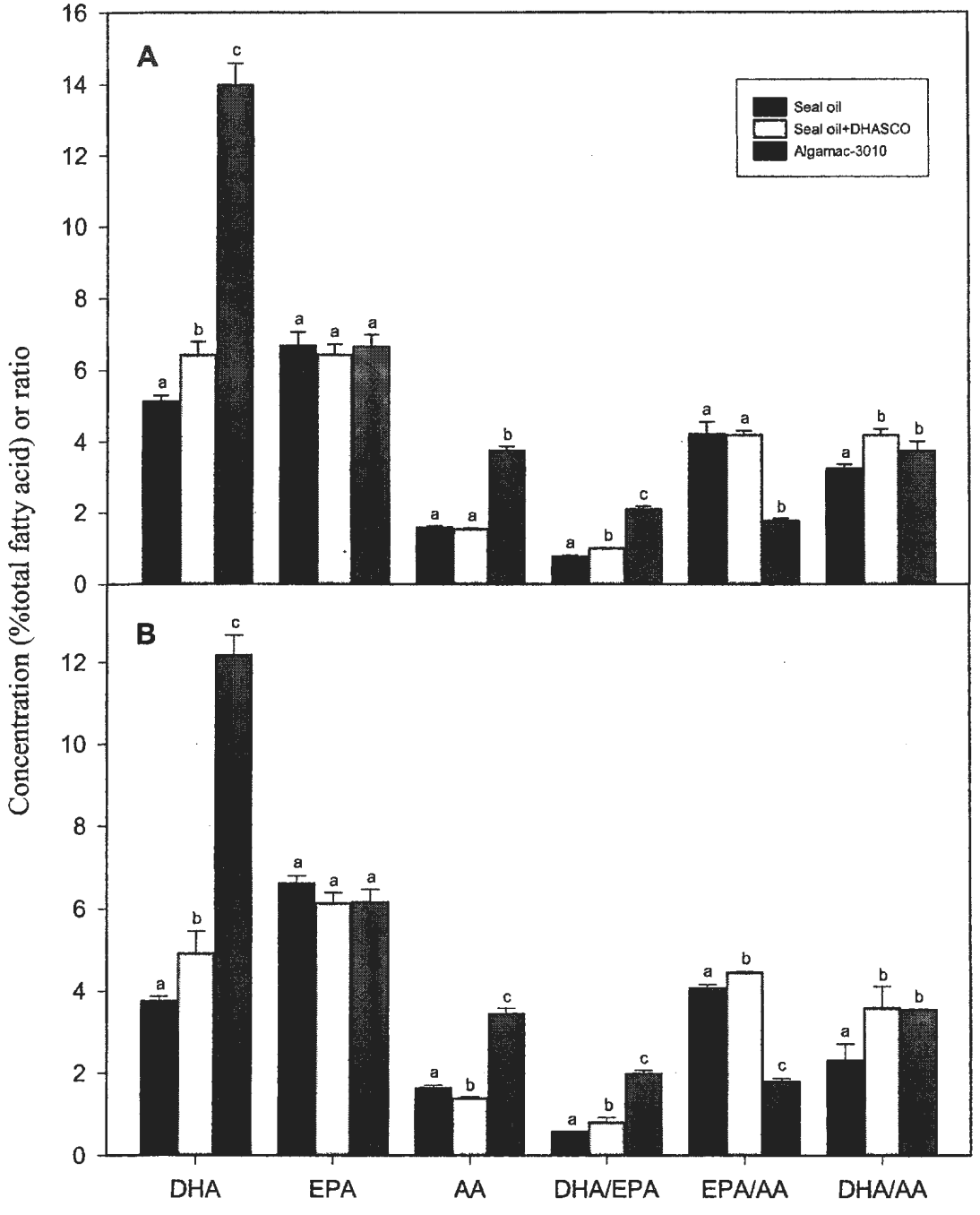

Fig. 4.3. The concentration of DHA, EPA, and AA and their respective ratio in the total lipid of normally (A) and abnormally (B) pigmented yellowtail flounder (*Limanda ferruginea*) fed live feeds enriched with seal oil, seal oil+DHASCO and algamac-3010. Vertical bars represent standard deviations, n = 3. Different letters (a,b,c) indicate significant difference at 0.05 level.

the content of AA in fish under Algamac feeding in the second experiment was much higher ($p<0.05$) than that of fish under seal oil and seal oil + DHASCO feeding regimes.

4.3.6 Fatty acid composition of abnormally pigmented yellowtail flounder juveniles

The fatty acid composition of the abnormally pigmented yellowtail flounder was, in general, similar to that of normally pigmented fish (Table 4.7, 4.8 and 4.9). Alpha-linolenic acid (18:3n-3) and 18:1n-9 were the most abundant fatty acids present in fish in both experiments. In the first experiment, 18:3n-3 and 18:1n-9 accounted for 17.2 to 21.3% and 12.3 to 16.0% of the fish fatty acids, respectively. The reverse was, however, evident in the second experiment where 18:3n-3 represented between 12.4 and 14.3%, and 18:1n-9 contributed 13.7 to 19.7% of the fish fatty acids.

As in the normally pigmented fish, SFAs in abnormally pigmented flounder were dominated by 16:0, which contributed 8.61 to 9.69% and 10.4 to 11.7% in the first and second experiments, respectively. Stearic acid (18:0) was also present in a considerable amount, particularly in the second experiment (4.16 – 4.66%). Other saturated fats were present at a much lesser amount. In the MUFA group, besides 18:n-9, there was appreciable amounts of 16:1n-7 and 18:1n-7 also present (3.12 – 6.97 and 4.72 – 6.30%, respectively) in the experimental fish. The relative contents of these fatty acids in the abnormally pigmented flounder were similar to those in their normally pigmented counterparts.

The PUFA in the abnormally pigmented flounder were, as in the normally pigmented fish, mainly of the n-3 family. The n-6 family was principally made up of 18:2n-6, except in the fish under Algamac treatment where 18:2n-6 and AA existed at a

Table 4.8. Fatty acid composition (%) of total lipids of abnormally pigmented yellowtail flounder (*Limanda ferruginea*) fed live feeds enriched with seal oil, menhaden oil and Algamac-3010 emulsions for 62 days.

Fatty acid	Yellowtail flounder fed on live feeds enriched with:					
	Seal oil		Menhaden oil		Algamac-3010	
	Mean	SD	Mean	SD	Mean	SD
12:0	0.21[a]	0.01	-	-	-	-
14:0	1.66[a]	0.11	1.36[b]	0.05	1.57[a]	0.16
14:1n-5	0.94[a]	0.03	0.91[a]	0.04	0.84[b]	0.03
15:0	0.50[a]	0.03	0.21[b]	0.02	0.23[b]	0.03
16:0	8.61[a]	0.47	9.36[b]	0.57	9.69[b]	0.23
16:1n-7	5.89[a]	0.25	6.97[b]	0.45	4.30[c]	0.34
16:2n-4	0.50[a]	0.04	0.52[a]	0.03	0.43[b]	0.03
16:3n-4	2.26[a]	0.16	1.31[b]	0.12	1.36[b]	0.12
16:4n-3	0.40[a]	0.02	0.44[a]	0.04	0.30[b]	0.03
17:0	0.53[a]	0.01	0.43[b]	0.05	0.54[a]	0.03
17:1	1.78[a]	0.06	2.18[b]	0.05	2.21[b]	0.08
18:0	2.77[a]	0.15	3.11[b]	0.14	2.97[ab]	0.21
18:1n-11	3.04[a]	0.11	1.04[b]	0.07	0.85[c]	0.05
18:1n-9	15.6[a]	1.09	16.0[a]	0.60	12.3[b]	0.20
18:1n-7	4.97[a]	0.46	5.70[b]	0.23	4.98[a]	0.33
18:2n-6	4.43[a]	0.10	4.75[b]	0.04	4.30[c]	0.09
18:3n-6	0.56[a]	0.01	0.60[a]	0.06	0.74[b]	0.01
18:3n-3	17.2[a]	1.41	20.7[b]	0.69	21.3[b]	0.54
18:4n-6	1.78[a]	0.05	0.41[b]	0.04	0.44[b]	0.04
18:4n-3	1.90[a]	0.14	0.66[b]	0.08	0.57[b]	0.04
20:0	1.10[a]	0.07	0.27[b]	0.02	0.31[b]	0.02
20:1n-11	0.71[a]	0.05	0.32[b]	0.00	0.28[b]	0.01
20:1n-9	1.16[a]	0.09	1.17[a]	0.12	0.52[b]	0.02
20:2n-6	0.11[a]	0.02	0.22[b]	0.03	0.22[b]	0.01
20:4n-6	1.71[a]	0.21	1.98[b]	0.14	3.74[c]	0.15
20:4n-3	1.39[a]	0.28	1.65[a]	0.27	1.67[a]	0.08
20:5n-3	6.23[a]	0.46	7.20[b]	0.60	6.20[a]	0.12
22:0	0.10[a]	0.00	0.11[a]	0.01	0.10[a]	0.00
22:1n-11	0.39[a]	0.01	-	-	-	-
22:4n-6	0.39[a]	0.01	-	-	-	-
22:4n-3	0.68[a]	0.04	0.86[b]	0.03	-	-
22:5n-3	2.29[a]	0.13	1.78[b]	0.17	0.99[c]	0.07
22:6n-3	5.21[a]	0.47	5.58[a]	0.39	10.9[b]	0.41
DHA/EPA	0.85[a]	0.06	0.78[a]	0.07	1.76[b]	0.04
EPA/AA	3.65[a]	0.06	3.64[a]	0.12	1.66[b]	0.13
DHA/AA	3.09	0.65	2.82	0.13	2.92	0.09

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. SD values < 0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Table 4.9. Fatty acid composition (%) of total lipids of abnormally pigmented yellowtail flounder (*Limanda ferruginea*) fed live feeds enriched with seal oil, seal oil+DHASCO and Algamac-3010 emulsions for 62 days.

Fatty acid	Yellowtail fed on live prey enriched with:					
	Seal oil		Seal oil + DHASCO		Algamac-3010	
	Mean	SD	Mean	SD	Mean	SD
14:0	1.08^{a}	0.02	1.34^{b}	0.02	1.24^{c}	0.03
14:1n-5	0.50^{a}	0.03	0.49^{ab}	0.05	0.44^{b}	0.01
15:0	0.26^{a}	0.01	0.25^{a}	0.04	0.32^{b}	0.01
16:0	10.4^{a}	0.28	10.9^{a}	0.17	11.7^{b}	0.88
16:1n-7	5.10^{a}	0.10	4.72^{b}	0.09	3.12^{c}	0.22
16:2n-4	0.45^{a}	0.01	0.43^{ab}	0.05	0.39^{b}	0.01
16:3n-4	0.74^{a}	0.03	0.75^{a}	0.07	0.71^{a}	0.08
16:4n-3	0.66^{a}	0.05	0.56^{b}	0.03	0.83^{c}	0.03
17:0	0.76^{a}	0.03	0.74^{ab}	0.06	0.68^{b}	0.04
17:1	0.89^{a}	0.09	0.67^{b}	0.06	0.94^{a}	0.06
18:0	4.16^{a}	0.16	4.20^{a}	0.40	4.66^{a}	0.58
18:1n-9	19.7^{a}	0.53	19.3^{a}	0.79	13.7^{b}	0.59
18:1n-7	6.30^{a}	0.26	6.00^{a}	0.46	4.72^{b}	0.20
18:2n-6	5.57^{a}	0.31	5.49^{a}	0.12	3.84^{b}	0.13
18:3n-6	0.69^{a}	0.04	0.68^{a}	0.03	0.72^{c}	0.03
18:3n-3	14.3^{a}	0.50	14.3^{a}	0.39	12.4^{b}	0.63
18:4n-6	2.82^{a}	0.19	3.09^{a}	0.33	2.43^{b}	0.03
18:4n-3	0.11^{a}	0.01	0.12^{a}	0.01	0.12^{c}	0.01
20:0	0.29^{a}	0.01	0.29^{a}	0.03	0.17^{b}	0.00
20:1n-11	0.34^{a}	0.01	0.35^{a}	0.03	0.30^{b}	0.02
20:1n-9	2.25^{a}	0.12	2.13^{a}	0.14	1.01^{b}	0.01
20:2n-6	0.31^{a}	0.00	0.27^{b}	0.02	0.33^{c}	0.01
20:4n-6	1.63^{a}	0.08	1.38^{b}	0.05	3.44^{c}	0.14
20:4n-3	1.86^{ab}	0.11	1.90^{a}	0.14	1.66^{b}	0.16
20:5n-3	6.61^{a}	0.18	6.13^{b}	0.26	6.16^{b}	0.31
22:0	0.33^{a}	0.01	0.34^{a}	0.02	0.37^{b}	0.02
22:1n-11	0.31^{a}	0.02	0.33^{a}	0.02	0.27^{b}	0.01
22:2n-6	0.16^{a}	0.02	0.19^{b}	0.02	0.19^{b}	0.01
22:4n-6	0.75^{a}	0.04	1.34^{b}	0.07	1.38^{b}	0.08
22:4n-3	0.79^{a}	0.04	0.93^{a}	0.31	4.33^{b}	0.31
22:5n-3	1.60^{a}	0.20	1.08^{b}	0.16	0.84^{c}	0.07
22:6n-3	3.75^{a}	0.12	4.91^{b}	0.54	12.2^{c}	0.49
DHA/EPA	0.57^{a}	0.00	0.80^{b}	0.12	1.98^{c}	0.09
EPA/AA	4.07^{a}	0.09	4.45^{b}	0.02	1.79^{c}	0.08
DHA/AA	2.31^{a}	0.04	3.58^{b}	0.53	3.54^{b}	0.01

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. SD values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHASCO, DHA-rich single cell oil; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

relatively similar concentration. In the n-3 family, the second most abundant fatty acids after 18:3n-3 was EPA followed by DHA, except in fish fed Algamac-enriched feeds where DHA existed as the second most abundant fatty acid. While the DHA content in abnormally pigmented juveniles was similar to that of normally pigmented flounder in the first experiment, its content in the abnormally pigmented fish under seal oil (3.75%) and seal oil+DHASCO (4.91%) feeding in the second experiment was much lower compared to that of the normally pigmented fish (5.13 and 6.42%, respectively). The DHA level in Algamac treatment was, however, similar between abnormally (12.2%) and normally (14.0%) pigmented fish. The content of EPA was found similar among feeding treatments, whereas the content of AA was higher ($p<0.05$) in fish fed Algamac-enriched prey compared to that of fish under the other feeding regimes (Fig. 4.2B and 4.3B).

4.3 Discussion

4.4.1 Live feeds

The results clearly showed that after enrichment, there was an increase in total lipid, and that the content of SFAs, MUFAs, PUFAs, n-6 and n-3 HUFAs in rotifers corresponded well with the levels in their diets, in agreement with the results from previous studies with rotifers (Rodriguez *et al.,* 1993, 1994, 1997; Rainuzzo *et al.,* 1994a). It was also evident that rotifers were able to accumulate DHA as efficiently as EPA, which is in contrast with previous results reported by Rainuzzo *et al.* (1994a) and Rodrigues (1994, 1997). These authors found that EPA was accumulated in rotifers better than DHA. They argued that, although the reason(s) for this different

accumulation is still not clear, it might have been due to the fact that rotifers are mainly freshwater filtering organisms, and since the majority of freshwater microalgae have EPA as the dominant n-3 HUFA, the enzymatic system of rotifers could be pre-adapted to incorporate EPA more readily than DHA. In addition, an increase in the rate of DHA catabolism compared to that of EPA might be operative. It is interesting that inclusion of 4.12% of DHASCO oil into seal oil increased the content of DHA from 6.86 to 11.2% and decreased the content of EPA from 5.83 to 3.89% of the total fatty acids, resulting in an increase of DHA/EPA from 1.18 to 2.88. This indicates that there was no competition in the incorporation of DHA and EPA into rotifers lipid, and that the levels of these fatty acids in rotifers were dependent on their levels in the diets.

The fatty acid composition of the feed sources was well reflected in the enriched rotifers but to a lesser extent in the *Artemia*. The incorporation of n-3 HUFA in rotifers was more efficient than in *Artemia*. Although the Algamac enrichment medium contained a low level of 18:3n-3, *Artemia* contained levels of that fatty acid approximately 14 – 19% of total fatty acids. Izquierdo *et al.* (1992) reported that the high content of 18:3n-3 in enriched *Artemia* was due to high levels of this fatty acid in the *Artemia* cysts (30% of total fatty acid in the present study). In Algamac-enriched *Artemia,* a relatively high level (2.32 – 3.16% dry wt.; 12 – 15% of the total fatty acids) of DHA was found, indicating that *Artemia* were able to incorporate high amount of DHA into their body lipids. Izquierdo *et al.* (1992) found levels of about 2.9% DHA (dry wt.) in *Artemia* fed oils containing 85% n-3 HUFA as ethyl esters, which was similar to the levels found in *Artemia* enriched with Algamac containing 28.85% n-3 HUFA in the

present study. Rainuzzo *et al.* (1994b) reported DHA content in *Artemia* enriched with copepod oil, halibut roe and purified oil emulsions between 5.57 and 29.9% of total fatty acids. Different enrichment methods, *Artemia* strain used and/or experimental conditions could explain the high variations of these results (Rainuzzo *et al.,* 1997).

The present experiment also showed that the presence of high amounts of EPA in the diets depressed the accumulation of DHA in *Artemia.* Unlike in rotifers, these two fatty acids posed a competition in their assimilation into *Artemia* lipids, and it appears that *Artemia* assimilates EPA better than DHA, if DHA is present in lower level than EPA in diets. However, when *Artemia* were given diets containing higher DHA than EPA levels, the assimilation of DHA was higher EPA. Therefore, the assimilation of any of these fatty acids into *Artemia* lipids depends on their dietary amounts. The DHA incorporation was always accompanied by an EPA increase, which may indicate some metabolic conversion of DHA to EPA by the nauplii during the enrichment process as reported previously by Watanabe (1993), Furuita *et al.* (1996) and Han *et al.* (2001). Han *et al.* (2000) reported that the levels of EPA, despite its dietary absence, increased in enriched *Artemia.* Similar results were also observed in the present study, and were particularly evident in *Artemia* enriched with Algamac emulsion. The content of EPA in Algamac and unenriched nauplii was low (0.93% and 2.82% of total fatty acids, respectively), but it exhibited a level between 4.13 and 6.43% in enriched *Artemia.* The presence of relatively high proportion of DPA (22:5n-3) in Algamac-fed *Artemia* (5.39%) and in uneaten *Artemia* (4.61%) previously enriched with Algamac (0.36%) emulsion confirmed the occurrence of DHA retroconversion, as discussed earlier.

4.4.2 Larvae

The analysis of fatty acid composition of yellowtail flounder larvae at 1- and 20-days post first feeding showed that 16:0, 18:1n-9 and DHA were the dominant fatty acids present. These results confirm the data reported by Knox *et al.* (1988) for rainbow trout; Watanabe *et al.* (1989) for red seabream and Koven *et al.* (1989), Mourente and Odriozola (1990) and Rodriguez *et al.* (1994) for gilthead seabream. The presence of high levels of these fatty acids in larval fish implies that they play an important role during larval development. From a marked reduction of 16:0 and DHA during the first 3-weeks of development and the relatively constant composition of the rest, it seems that these two fatty acids are the greatest source of larval stored energy and generally primarily catabolized. Larval fish maintained, however, 16:0 within a narrow range (around 10% of total fatty acids), regardless of the high variability of this fatty acid in the diets. Tandler *et al.* (1989) suggested that DHA may be utilized as energy during larval development or converted to other physiologically important substances. The results of the present study seem to indicate that during the first 3 weeks, its function would be more structural than catabolic. This is supported by the fact that DHA, as well as EPA and AA, levels in larvae did not decrease below the levels in feeds during this period. Koven *et al.* (1989) have shown that, in starved *Sparus auratus*, n-3 series of fatty acids were clearly conserved at the expense of other fatty acid groups. This fact suggests that the EFAs are more valuable as essential components of biological membranes than as energy stores. These fatty acids play many roles in embryos and larvae, and are particularly important for nervous system development (Izquierdo *et al.,* 1992; Devresse

et al., 1994; Dhert *et al.,* 1994; Bell *et al.,* 1995; Estévez and Kanazawa, 1995, 1996; Kanazawa, 1995; Estévez *et al.,* 1997; Rainuzzo *et al.,* 1997; Sargent *et al.,* 1997, 1999).

Similarly, the much higher levels of 16:1n-7 and 18:1n-9 in the diet compared to those in 20-days post first feeding larvae indicated a preferential use of MUFAs as substrate for catabolism by larval yellowtail flounder and is consistent with previous findings for rainbow trout (Kiessling and Kiessling, 1993). In goldfish, larvae from higher-quality eggs were able to designate MUFAs for catabolism better than those from lower-quality eggs (Wiegand *et al.,* 1991). MUFAs are also important in structural lipids. For example, the insertion of a MUFA, particularly 18:1n-9, in the *sn-1* position of phosphatidylethanolamine (PE) is an important adaptation to permit adequate membrane fluidity in cool conditions (Dey *et al.,* 1993; Buda *et al.,* 1994), as is the increase in the relative abundance of PE compared with phosphatidylcholine (PC) (Hazel, 1989). Bodies of newly hatched goldfish raised at 13 °C had lower levels of 18:1n-9 in their total lipids than those raised at 22 °C; the former, however, had higher levels of PUFAs (Wiegand *et al.,* 1991).

The n-3 HUFA content in fish increased proportionally to the n-3 HUFA content in live feeds. In all fish groups, the levels of EPA, DHA and AA also increased as these fatty acids increased in live feeds. The elevation of EFA content in rotifers increased the EFA level in 20-days post first feeding larvae, suggesting a direct relationship. The levels of DPA (22:5n-3) in juveniles reflected, to some extent, that of the diets and increased from 1.77% in 1-day post first feeding to 1.83 – 3.72% in 20-days post first feeding larvae. Considering the lower level of DPA in 1-day post first feeding larvae, the

presence of this fatty acid in rotifers and larval fish, which fed on them at a comparable level, suggested that an accumulation of DPA had taken place in larvae. No Δ4 desaturase activity, or a very low activity, occurred in yellowtail flounder to convert DPA to DHA, as has been suggested for marine fish (Sargent *et al.,* 1989). Evidence is accumulating that desaturation, rather than elongation, is limiting in the formation of DHA in marine fish (Tocher, 1993). Similarly, Rodriguez *et al.* (1997) reported that larval gilthead seabream receiving more EPA in the diet accumulated more DPA (22:5n-3) in their neutral lipid compared to those given higher DHA/EPA ratios.

The results of the present study indicated that the assimilation level of DHA into yellowtail flounder body lipid was higher than EPA, which contradicts with the results of Rodriguez *et al.* (1994). It appeared that this contradiction arose from the fact that both studies administered diets having very different DHA/EPA ratios. In this study, the enriched rotifers had a DHA/EPA ratio of 1 – 8, whereas Rodriguez *et al.* (1994) used rotifers with a DHA/EPA ratio of 0.34 – 0.52. It is, therefore, logical to conclude that the assimilation of DHA and EPA into larval fish depends on the amount of each of these fatty acids in the diet. This difference in the assimilation rate could be due to a competitive effect between these two fatty acids. Izquierdo *et al.* (1990) suggested a selective incorporation of DHA into the phospholipids of *Pagrus major* larvae. It has also been shown that fish oil fatty acids compete more efficiently for incorporation into membrane phospholipids than other lipid sources (Nilsson *et al.,* 1992). The better assimilation of DHA and the constant levels of EPA in larvae resulted in increased DHA/EPA ratios. A number of studies have suggested that a key factor in larval

development is the ratio (2 or higher) of DHA to EPA (Devresse *et al.,* 1994; Dhert *et al.,* 1994; Rainuzzo *et al.*, 1994b; Sargent *et al.,* 1997), while others indicate that absolute levels of these fatty acids may be more important than their ratios (Næss and Lie, 1998).

4.4.3 Juvenile fish

The total lipid content of yellowtail flounder was found to be similar between normally and abnormally pigmented counterparts. This indicates that normally and abnormally pigmented fish possess similar capacity to accumulate dietary lipid in their tissues. However, the total lipid contents of fish were also affected by the dietary lipid levels. Fish fed seal oil-enriched *Artemia*, which contained the highest lipid content, showed significantly higher total lipid content compared to those of fish under the other feeding regimes.

The levels of SFAs and MUFAs in fish were generally lower than their dietary levels, whereas that of PUFAs were higher, indicating that dietary SFAs and MUFAs were the main sources of energy in yellowtail flounder. The present experiment demonstrated, however, that there was no significant difference in the relative levels of SFAs, MUFAs and PUFAs in the flounder body lipid between normally and abnormally pigmented fish. This indicates that the degree of unsaturation in lipids of normally and abnormally pigmented flounder is similar, and that the degree of unsaturation is not diet- or pigmentation type-dependent. These findings are in contrast to those of Estévez and Kanazawa (1996) who found a higher degree of unsaturation in normally pigmented fish.

The overall fatty acid composition of total lipids of normally and abnormally pigmented yellowtail flounder juveniles fed live feeds enriched with different sources of

oil emulsions was generally similar, although some variations existed. The fatty acid compositions of most marine fish lipids are generally similar. The main saturated fatty acid is almost always 16:0, while the principal monounsaturated fatty acid is almost always 18:1n-9 and the main polyunsaturated fatty acids are usually DHA and EPA. In this study, the only marked difference was the proportions of DHA and AA in fish fed Algamac-enriched diets, which were much higher than those in fish under different feeding regimes. These results confirm the findings of Estévez and Kanazawa (1996) who reported that the overall fatty acid profile in neural tissues of Japanese flounder raised on different diets was very similar in normally pigmented and malpigmented fish. These authors also found that neural tissues of albino fish had less EPA and higher DHA/EPA ratios than those of normal pigmented fish, similar to the results of the second experiment of the present study. In contrast, McEvoy *et al.* (1998) and Estévez *et al.* (1999) found no significant difference in the DHA/EPA ratios between tissues from normally and abnormally pigmented halibut juveniles fed *Artemia* enriched with the same diets, which also agrees with the results from the first experiment of this study with yellowtail founder. Estévez *et al.* (1999) explained these apparent anomalies by hypothesizing that the DHA/EPA ratio is not *per se* fundamental to pigmentation in the sense that, given a sufficiency of dietary DHA, the optimum dietary level of EPA is then not a function of dietary DHA but of dietary AA. Therefore, optimization of not only the absolute dietary amounts but also the balance of DHA, EPA and AA in the nutrition of larval fish is important. The dietary requirement for any of these three EFA is not an

independent function, but is influenced by the levels of the other two fatty acids present (Estévez *et al.,* 1999).

As in the 20-days post first feeding larvae, juvenile yellowtail flounder also maintained 16:0 within a narrow range (8.61 – 11.7% of total fatty acids), regardless of the diets and pigmentation types. No significant difference existed in the level of this fatty acid, as well as the level of 18:0, between normally and abnormally pigmented flounder. This is in contrast to the findings of Estévez and Kanazawa (1996) who reported that 16:0 and 18:0 were higher in the body neutral and polar lipid of unpigmented Japanese flounder. It seems that these fatty acids, particularly 16:0, also plays important roles not only as energy reserves, but also as components of membrane structures. For example, the insertion of 16:0 or other saturated fatty acids in the *sn-1* position of a phospholipid molecule decreases the fluidity of membranes, which is important for adequate membrane fluidity in warm conditions.

When fish were fed with diets containing low levels of DHA and AA (*Artemia* enriched with seal and menhaden oil), the levels of these EFA were higher in the fish compared to those in the diets, indicating accumulation of both DHA and AA in fish tissues. On the other hand, the levels of EPA in flounder were similar to the level in their diets and maintained in a narrow range (5.84 – 7.20%). From this experiment, it was therefore clear that even when diets contained over 7% EPA, the level of this fatty acid was still lower in fish. It seems that assimilation of DHA into fish lipids is more efficient when its dietary level was lower than that of EPA. Watanabe *et al.* (1989) have shown that the level of assimilation of DHA was much higher than EPA. However, when the

level of DHA in the diet approached the level of EPA, there seems to be a competition between them, resulting in a lower DHA assimilation. This was evident from the fact that the level of DHA did not exceed that of EPA even when their dietary levels were similar, except when diets contained far higher DHA than EPA, as in the case of fish fed Algamac-enriched prey. This supports the hypothesis that DHA and EPA are competitive and suggests that the EFA requirements of marine fish larvae depend on the DHA/EPA ratio in the diet. Iijima *et al.* (1998) have shown that competition among DHA, EPA and AA could begin from the initial digestion processes, since bile-salt activated lipase (BAL) showed a higher affinity for EPA or AA than DHA esterified to neutral lipids. The presence of such competitive interaction among EFAs implies that, in order to estimate the dietary requirement, consideration of not only their absolute amounts but also their proportion in the diets is very important. It has also been shown that starved larvae tend to conserve DHA and AA, which are associated with neural functions (Rainuzzo *et al.,* 1994b). Similarly, adult turbot fed a diet deficient in PUFAs selectively retained both DHA and AA at the expense of EPA, suggesting that DHA and AA have more important biochemical functions than EPA in the phospholipids (PL) (Bell *et al.,* 1985). Castledine and Buckley (1982) suggested that conservation of fatty acids, such as DHA, during starvation is not a result of their incorporation in a metabolically inert "structural" phospholipid pool but, rather, a result of their selective re-utilization during the course of membrane biogenesis and turnover.

The levels of 16:1n-7, 18:1n-9, 18:1n-7, 18:2n-6 and 18:3n-3 remained lower in fish compared to those in their diets. It seems that these fatty acids and to a lesser extent

18:2n-6, are the main source of energy in yellowtail flounder juveniles. Since there was essentially no change in the levels of 18:4n-3 and EPA in fish, perhaps no synthesis of EPA from 18:3n-3 or retroconversion of EPA to 18:3n-3 took place, although some retroconversion activity from 20:5n-3 to 18:4n-3 has been reported in turbot cell lines (Tocher and Mackinlay, 1990). Similarly, there was no clear evidence that elongation and desaturation of EPA to DHA or retroconversion of DHA to EPA took place, since the level of 22:5n-3 was essentially unchanged. Henderson *et al.* (1998) reported that elongation of 22:5n-3 to 24:5n-3 in trout liver microsomes appeared to proceed at a greater rate than the elongation of 20:5n-3 to 22:5n-3. This is consistent with 22:5n-3 being a minor PUFA in fish lipids in general (Henderson and Tocher, 1987). Brenner (1989) has reported the rate of fatty acid desaturation within any unsaturated fatty acid family is generally slower than the rate of elongation. However, Olsen and Ringo (1992) have also reported that feeding a commercial diet to fish virtually diminished elongation and desaturation fatty acids in both polar and neutral lipid classes, showing the importance of diet on lipogenic processes. Similarly, Buzzi *et al.* (1996) found that the presence of fish oil containing preformed EPA and DHA in the diet of trout results in a marked decrease in the rates of formation of DHA from 18:3n-3 in hepatocytes isolated from the fish. Olsen and Ringo (1992) also found that, under farm conditions, Arctic charr are incapable of elongating and desaturating dietary shorter chain PUFA. Whether this is caused by the high level of long chain PUFA in the diet or some other nutritional factors is unclear. It is possible that PUFA, including DHA, can also exert post-transcriptional influences on desaturase activity by changing the fatty acid composition of

the phospholipids in the membranes surrounding the enzymes, thus regulating the enzyme's activity (Giron *et al.,* 1996). The ability of long chain n-3 PUFAs to regulate the transcription of genes coding for lipogenic enzymes is well documented (Clarke and Jump, 1994).

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Chapter 5

Effect of Varying Enrichment Concentration and Period on Lipid Content and Fatty Acid Composition of Rotifers (*Brachionus plicatilis*) Enriched with Different Oil Emulsions

5.1 Introduction

One of the important starter live feeds used in fish larviculture is the marine rotifer (*Brachionus plicatilis*). Rotifers are used as a live feed during the initial feeding of several marine fish, due to their size (130-300 μm) and the availability of large quantities by mass cultivation (Lie *et al.,* 1997). However, the low nutritional value of this live feed is a major problem, and several cultivation techniques, including feeding with algae and artificial diets, have been used to improve their nutritional quality (White and Nagata, 1990; Nagata and White, 1992; Caric *et al.,* 1993; Fernandez-Reiriz *et al.,* 1993; Olsen *et al.,* 1993a,b). The successful development of commercial marine finfish aquaculture has been made possible by several improvements in the production techniques of this live feed (Candreva *et al.*, 1996; Dehasque *et al.*, 1998). The nutritional aspects of rotifers have received major attention in larviculture and several commercial products have been launched to increase the lipid and vitamin content in rotifers (Coutteau and Sorgeloos, 1997). Although it is technically possible to produce rotifers of high nutritional value, their quality is often far from optimal due to their low hygienic and overall condition (low swimming velocity, low reproduction rate) (Dhert *et al.*, 2001).

Cultured rotifers are generally low in their content of eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). These are essential fatty acids and hence a common practice is to enrich these live prey with emulsions of marine oils. The short-term exposure to oil emulsions results in rotifers-encapsulated lipid with high EPA and DHA levels. However, rotifers are prone to fast loss of gut content and show a distortion in their protein/lipid balance (Dhert *et al.*, 2001). Short-term enrichment (generally less than 8 h) has the advantage of being fast and flexible, but very often produces lower quality rotifers with a too high lipid content (Dhert *et al.*, 1990; Støttrup and Attramadal, 1992) and poor hygienic quality (Dhert *et al.,* 2001). Also, transfer of oil to larval rearing tanks with consequent loss of water quality and associated problems of larval viability have been reported (Foscarini, 1988). Emulsions with phospholipids have also been used as a more efficient fatty acid source for fish (McEvoy *et al.*, 1996; Coutteau *et al.*, 1997), but they are immediately broken down in rotifers (Dhert *et al.*, 2001). In addition, the retention time of the nutrients, which are mainly accumulated in the digestive tract of the rotifers, is very short and can create problems when the rotifers are not eaten immediately by fish larvae (Dhert *et al.*, 2001).

Rotifers are not selective for the uptake or catabolism of highly unsaturated fatty acids, therefore, high HUFA levels can be accumulated without problem (Dhert *et al.*, 2001). DHA, an essential fatty acid that accumulates in the brain of fish during early development where it increases neural function (Bell *et al.*, 1995), is especially easily incorporated in rotifers, unlike *Artemia*, which preferentially catabolizes this fatty acid (Dhert *et al.*, 1993; Estévez *et al.*, 1999). In line with the important role of DHA in

neural function, the feeding with DHA-enriched rotifers is often prolonged in flatfish cultures where the enrichment at an early stage has been successful in improving pigmentation (Miki *et al.*, 1990; Kanazawa, 1993; Reitan *et al.*, 1994). However, further investigations are still needed to reveal the optimum mixture of DHA with EPA and arachidonic acid (AA) in the nutrition of larval fish (Estévez *et al.*, 1999). The present experiment was devised to determine how different enrichment concentrations and periods affect the total lipid, fatty acid composition and essential fatty acid levels in rotifers (*Brachionus plicatilis*) enriched with various oil emulsions.

5.2 Materials and methods

Newly harvested rotifers (*Brachionus plicatilis*) from stock tanks were divided into 48 batches for enrichment with different concentrations of oil emulsions at different enrichment periods. Each batch was given an oil emulsion of either seal blubber oil (AMi Company, St. John's, NF), menhaden oil (Omega Protein, Reedville, VA), Algamac-3010 (Aquafauna Bio-Marine, Inc., Hawthorne, CA) or DHASCO (Martek Bioscience Corp., Columbia, MD). The concentrations of seal oil and menhaden oil were 0.2, 0.4, 0.6 and 0.8 g/million of rotifers. The Algamac-3010 emulsion concentrations were 0.2, 0.3, 0.4 and 0.5 g/million of rotifers, whereas those of DHASCO were 0.1, 0.2, 0.3, and 0.4 g/million of rotifers. Emulsions were prepared by vigorously mixing each enrichment medium with 200 mL seawater and 0.2 g raw egg yolk (as emulsifier) for approximately 3 min. Enrichment with each type of emulsion or concentration was carried out in triplicate.

Enrichment of rotifers was conducted 6 h post-harvest for 6, 12, 18 and 24 h in 48 4-L capacity of buckets (4 emulsion concentrations x 4 enrichment periods x 3 replicates). The density of rotifers in the enrichment media was 220/mL. All emulsions were divided into two portions; the second portion was offered at 3, 6, 9 and 12 h after the first portion was given, for enrichment periods of 6, 12, 18 and 24 h, respectively. At the end of each enrichment period, enriched rotifers were harvested through a 60-μm-mesh screen, washed with filtered seawater to remove traces of oils, and subsequently washed with filtered freshwater to remove salt. Enriched rotifers were then blotted with paper towels, transferred to 10 mL glass vials and stored under nitrogen at – 20°C until used for analysis.

Lipid extraction, total lipid determination, fatty acid composition analysis and statistical analysis were carried out following the procedures described previously in Chapter 2.

5.3 Results

5.3.1 Total lipid content of enriched rotifers

The results showed that the enrichment concentration, period and the source of enrichment medium exerted some significant effects on the total lipid content of rotifers (Table 5.1). Statistical analysis of data using two-way ANOVA indicated that emulsion concentration affected ($p \leq 0.02$) the total lipid content of rotifers, except for those rotifers enriched with DHASCO emulsion ($p = 0.15$). The effect of enrichment period

Table 5.1. Total lipid content (%, wet wt.) of rotifers (*Brachionus plicatilis*) enriched with various oil emulsions at different concentrations and enrichment periods.

Enrichment period (h)	Emulsion concentration (g/million rotifers)							
	0.2		0.4		0.6		0.8	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Initial	3.12 ± 0.13							
Seal oil								
6	$3.12^{a,x}$	0.10	$3.47^{b,x}$	0.05	$3.37^{b,x}$	0.07	$3.53^{b,x}$	0.05
12	3.47^{y}	0.08	3.41^{x}	0.07	3.33^{x}	0.10	3.36^{x}	0.11
18	3.31^{xy}	0.13	3.37^{x}	0.10	3.47^{x}	0.12	3.50^{x}	0.07
24	3.82^{z}	0.07	3.78^{y}	0.15	3.89^{y}	0.09	3.87^{y}	0.06
Menhaden oil								
6	$3.22^{a,x}$	0.07	$3.35^{ab,xz}$	0.06	$3.40^{b,xy}$	0.04	$3.45^{b,xy}$	0.06
12	$3.36^{a,x}$	0.08	$3.19^{a,x}$	0.11	$3.23^{a,x}$	0.10	$3.62^{b,xz}$	0.08
18	3.62^{y}	0.08	3.60^{y}	0.06	3.48^{yz}	0.08	3.41^{y}	0.10
24	$3.41^{a,xy}$	0.11	$3.45^{ab,yz}$	0.13	$3.69^{b,z}$	0.09	$3.73^{c,z}$	0.07
Algamac-3010	0.2		0.3		0.4		0.5	
6	2.85^{x}	0.11	3.03^{x}	0.12	3.10^{x}	0.13	3.07^{x}	0.20
12	$3.11^{a,xy}$	0.06	$3.35^{ab,y}$	0.08	$3.30^{ab,xy}$	0.14	$3.41^{b,y}$	0.10
18	$3.35^{a,yz}$	0.10	$3.27^{a,xy}$	0.17	$3.36^{a,xy}$	0.07	$3.79^{b,z}$	0.09
24	3.41^{z}	0.14	3.53^{y}	0.09	3.49^{y}	0.19	3.66^{yz}	0.07
DHASCO	0.1		0.2		0.3		0.4	
6	3.17^{a}	0.07	3.28^{a}	0.08	3.47^{b}	0.07	3.33^{ab}	0.07
12	3.31	0.09	3.35	0.10	3.40	0.13	3.46	0.06
18	3.48	0.11	3.32	0.17	3.36	0.14	3.35	0.12
24	3.39	0.22	3.55	0.12	3.50	0.18	3.58	0.19

Analyses were carried out in triplicates. SD, standard deviation; DHASCO, DHA-rich single cell oil.
Values in each row for each emulsion with different superscripts (a,b,c) are different ($p<0.05$) from one another.
Values in each column for each emulsion with different superscripts (x,y,z) are different ($p<0.05$) from one another.

was, however, significant in all enrichment treatments. A significant interaction ($p < 0.001$) between the enrichment concentration and the enrichment period was observed only in seal oil and menhaden oil treatments (Table 5.2).

Rotifers enriched with seal oil emulsion contained total lipid between 3.12 and 3.89% on a wet weight basis. The lowest lipid content was found in rotifers enriched with the lowest emulsion concentration (0.2 g/million) at the shortest enrichment period (6 h), while the highest content was observed in rotifers enriched with the higher emulsion concentration (0.6 and 0.8 g/million) at the longest enrichment period (24 h). No difference ($p>0.05$) was observed in the total lipid contents of rotifers enriched with different emulsion concentrations at the enrichment period of 12, 18 and 24 h. Some differences ($p<0.05$), however, existed among enrichment periods for the same emulsion concentration, with rotifers enriched for 24 h consistently showing higher ($p<0.05$) lipid contents. Regression analysis showed a significant correlation ($r = 0.727$; $p < 0.001$) between total lipid content and the emulsion concentration and enrichment period. The total lipid content of rotifers enriched with seal oil can be predicted using the following equation: $Y = 3.044 + 0.208C + 0.024P$, where Y = lipid content (% wet wt.), P = enrichment period (h), and C = emulsion concentration (g/million prey).

In the menhaden oil enrichment, the effects of emulsion concentration and enrichment period on the total lipid contents of rotifers were slightly different from those observed in seal oil enrichment, although the lipid contents of rotifers under the two enrichment media were similar. The menhaden oil-fed rotifers contained between 3.19

Table 5.2. Results of two-way ANOVA of the effects of enrichment concentrations and periods on the total lipid and essential fatty acid (EFA) contents of rotifers (*Brachionus plicatilis*) enriched with various oil emulsions.

Concentration and period	Oil emulsion			
	Seal oil	Menhaden oil	Algamac-3010	DHASCO
	Total lipid			
Concentration	$p = 0.01$	$p < 0.001$	$p < 0.001$	$p = 0.15$
Period	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p = 0.01$
Interaction	$p < 0.001$	$p < 0.001$	$p = 0.05$	$p = 0.16$
	DHA			
Concentration	$p < 0.001$	$p < 0.001$	$p = 0.01$	$p < 0.001$
Period	$p < 0.001$	$p < 0.001$	$p = 0.03$	$p < 0.001$
Interaction	$p < 0.001$	$p < 0.001$	$p = 0.11$	$p < 0.001$
	EPA			
Concentration	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p = 0.21$
Period	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Interaction	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
	AA			
Concentration	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Period	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Interaction	$p = 0.02$	$p < 0.001$	$p < 0.001$	$p < 0.001$

DHASCO, DHA-rich single cell oil. DHA, docosahexaenoic acid; EPA, eicosahexaenoic acid; AA, arachidonic acid. Significant difference was considered at 5% of probability level.

and 3.73% of lipids (wet wt.) (Table 5.1), and increased with emulsion concentration and enrichment period, although not always in a significant ($p>0.05$) manner. Linear regression analysis indicated a significant correlation ($r = 0.610$; $p < 0.001$) between the total lipid content of rotifers and the enrichment period (P) and concentration (C) of enrichment medium. The lipid content of rotifers enriched with menhaden oil can be predicted using the following regression equation: $Y = 3.122 + 0.255C + 0.013P$.

The total lipid content of Algamac-enriched rotifers ranged from 2.85 to 3.79% (wet wt.) (Table 5.1). Although an increase in emulsion concentration resulted in an increase in the total lipid content of rotifers, this increase was not significant ($p>0.05$). Increasing the enrichment period, however, resulted in a higher ($p<0.05$) lipid content. Multiple linear regression also showed the existence of a significant correlation ($r = 0.835$; $p < 0.001$) between total lipid content of Algamac-enriched rotifers and the enrichment period (P) and concentration (C). The total lipid content of rotifers may be predicted from the equation $Y = 2.572 + 0.927C + 0.028P$.

For the DHASCO-enriched rotifers, the total lipid content was between 3.17 and 3.58% (wet wt.) (Table 5.1). In general, no differences ($p>0.05$) existed in the total lipid content regardless of the emulsion concentration and enrichment period. However, a significant correlation ($r = 0.505$; $p = 0.001$) existed between these parameters, the total lipid content of rotifers enriched with DHASCO can be calculated from the equation $Y = 3.164 + 0.360C + 0.009P$.

5.3.2 Fatty acid composition of total lipid of rotifers

The detailed fatty acid composition of rotifers enriched with various oil emulsions at different concentrations and enrichment periods is given in Appendices 5.1 – 5.16.

5.3.2.1 Saturated fatty acids (SFAs)

The saturated fatty acids (SFAs) family was composed primarily of palmitic acid (16:0), ranging from 5.52% in seal oil- to 23.65% of the total fatty acids in menhaden oil-enriched rotifers. The content of this fatty acid generally increased ($p < 0.05$) with increasing concentration of emulsion and enrichment period. Both myristic (14:0) and stearic (18:0) acids were found at much lower concentrations compared to that of 16:0. The content of 14:0 ranged from 2.63% in seal oil- to 9.91% in DHASCO-enriched rotifers, whereas that of 18:0 ranged from 1.78% in DHASCO- to 5.53% in menhaden oil-fed rotifers. Other SFAs were present in negligible amounts. In general, the proportion of each saturated fatty acid varied ($p < 0.05$) among the enrichment concentrations and periods.

5.3.2.2 Monounsaturated fatty acids (MUFAs)

The principal components of monounsaturated fatty acids (MUFAs) of seal oil-enriched rotifers were 18:1n-9 and 16:1n-7 acids, regardless of the concentration, enrichment period and the source of emulsions. Thus, 18:1n-9 and 16:1n-7 fatty acids contributed 7.86 – 31.1 and 7.99 – 21.0% of the total fatty acids, respectively. Fatty acid 18:1n-7 and 20:1n-9 were also found in appreciable proportions of 2.17 – 4.69% and 2.10

– 6.88%, respectively, in seal oil-enriched rotifers. All other MUFAs contributed negligible amounts to the total fatty acids of rotifers. The proportion of each fatty acid, in general, varied ($p < 0.05$) among different enrichment concentrations as well as periods of enrichment for each emulsion source, although some similarities ($p > 0.05$) were observed.

5.3.2.3 Polyunsaturated fatty acids (PUFAs)

The polyunsaturated fatty acids (PUFAs) of enriched rotifers were dominated by two essential fatty acids, DHA (22:6n-3) and EPA (20:5n-3). The proportion (% total fatty acids) of DHA was 3.94 – 8.29% in seal oil-, 4.58 – 9.27% in menhaden oil-, 24.6 – 26.8% in Algamac- and 22.0 – 28.5% in DHASCO-fed rotifers. The DHA content varied ($p<0.05$) among concentrations and periods of enrichment for each enrichment source, except among concentrations in the seal oil and Algamac treatments at 6 h enrichment period. EPA attained levels of 4.01 – 7.44% and 4.96 – 9.57% in seal oil and menhaden oil feedings, respectively; it exhibited, however, a much lower content in Algamac- (2.98 – 4.05%) and DHASCO- (1.18 – 1.95%) enriched rotifers. The contents of EPA varied ($p < 0.05$) among different enrichment concentrations and periods for each emulsion source, although some similarities ($p > 0.05$) were also observed. Both α–linolenic acid (18:3n-3) and docosapentaenoic acid (DPA, 22:5n-3) were also present in appreciable proportions. While the content of the former in the seal oil feeding dropped drastically from 3.05 and 3.87% in the 6 and 12 h to only 0.47 and 0.66% in the 18 h and 24 h enrichment periods, respectively, this fatty acid remained low (< 1.5%) in the menhaden

oil, Algamac and DHASCO treatments. The content of 22:5n-3 in seal oil-fed rotifers was 2.20 – 4.84% and increased with the duration of enrichment, although the highest values were found in the 18 h period. In menhaden oil feeding, 22:5n-3 attained levels of 1.24 – 2.59% of the total fatty acids, but contributed negligible amounts to the total fatty acids of Algamac- and DHASCO-fed rotifers (<1 %). The contents of these minor fatty acids varied according to the enrichment concentration and period, although a few similarities ($p > 0.05$) were observed.

Of the n-6 series of PUFAs, 18:2n-6 occurred at the highest proportion, ranging from 2.80 to 4.54% in seal oil-, 1.48 to 3.32% in menhaden oil-, 2.14 to 2.97% in Algamac- and 1.94 to 2.96% in DHASCO-fed rotifers. Arachidonic acid (20:4n-6) was present at very low levels under all emulsion concentrations, enrichment periods and emulsion sources. Its proportion ranged from 0.69 to 1.13% in seal oil, 0.63 to 1.46% in menhaden oil, 1.65 to 2.19% in Algamac and 0.37 to 0.53% in DHASCO feeding. γ–linolenic acid (18:3n-6) was present in seal oil enriched rotifers at proportions between 1.39 and 1.91%, whereas in rotifers enriched with menhaden oil, Algamac and DHASCO, its proportion was generally less than 1% of the total fatty acids. Fatty acid 18:4n-6 was found at a level between 1.19 and 2.55% in menhaden oil and 1.65 and 2.19% in Algamac feeding, while in seal oil and DHASCO feeding treatments its content was generally less than 1% of the total fatty acids. The content of each of the n-6 fatty acids in all feeding treatments generally varied ($p < 0.05$) among the enrichment concentrations and periods.

5.3.2.4 Essential fatty acid (EFA) ratio

The ratio of DHA/EPA in rotifers enriched with seal oil generally increased, although not always significantly, as the concentration of oil emulsion increased, but was generally unaffected by the enrichment period (Fig. 5.1). In contrast, the EPA/AA and DHA/AA ratios decreased with increasing emulsion concentration. Although some significant influences of enrichment period were observed on the EPA/AA and DHA/AA, the pattern of these effects was not clear. In menhaden oil-fed rotifers, the DHA/EPA ratio was essentially unaffected by the enrichment concentration and period (Fig. 5.2). Although the DHA/EPA ratios in rotifers enriched for 6 and 24 h tended to be higher than in rotifers enriched for 12 and 18 h, these differences were not always significant. On the other hand, both EPA/AA and DHA/AA ratios were higher ($p<0.05$) in the 12 and 24 h compared to the 6 and 18 h enrichment periods, and tended to increase with increasing emulsion concentration. While both the DHA/EPA and DHA/AA ratios increased slightly, but not significantly, with increasing enrichment concentration, the EPA/AA ratios in rotifers enriched with Algamac remained relatively unchanged (Fig. 5.3). Both the enrichment concentration and period did not affect the EFA ratios, except in the lowest emulsion concentration (0.2 g/million) where a significant effect of enrichment period was observed. In the DHASCO-enriched rotifers, the DHA/EPA ratio remained relatively unchanged among treatments, except in the prey fed with 0.4 g oil where the 12 h enrichment period showed a higher ($p<0.05$) value than those of the prey fed for 6, 18 and 24 h (Fig. 5.4). Similarly, the EPA/AA and DHA/AA ratios were generally

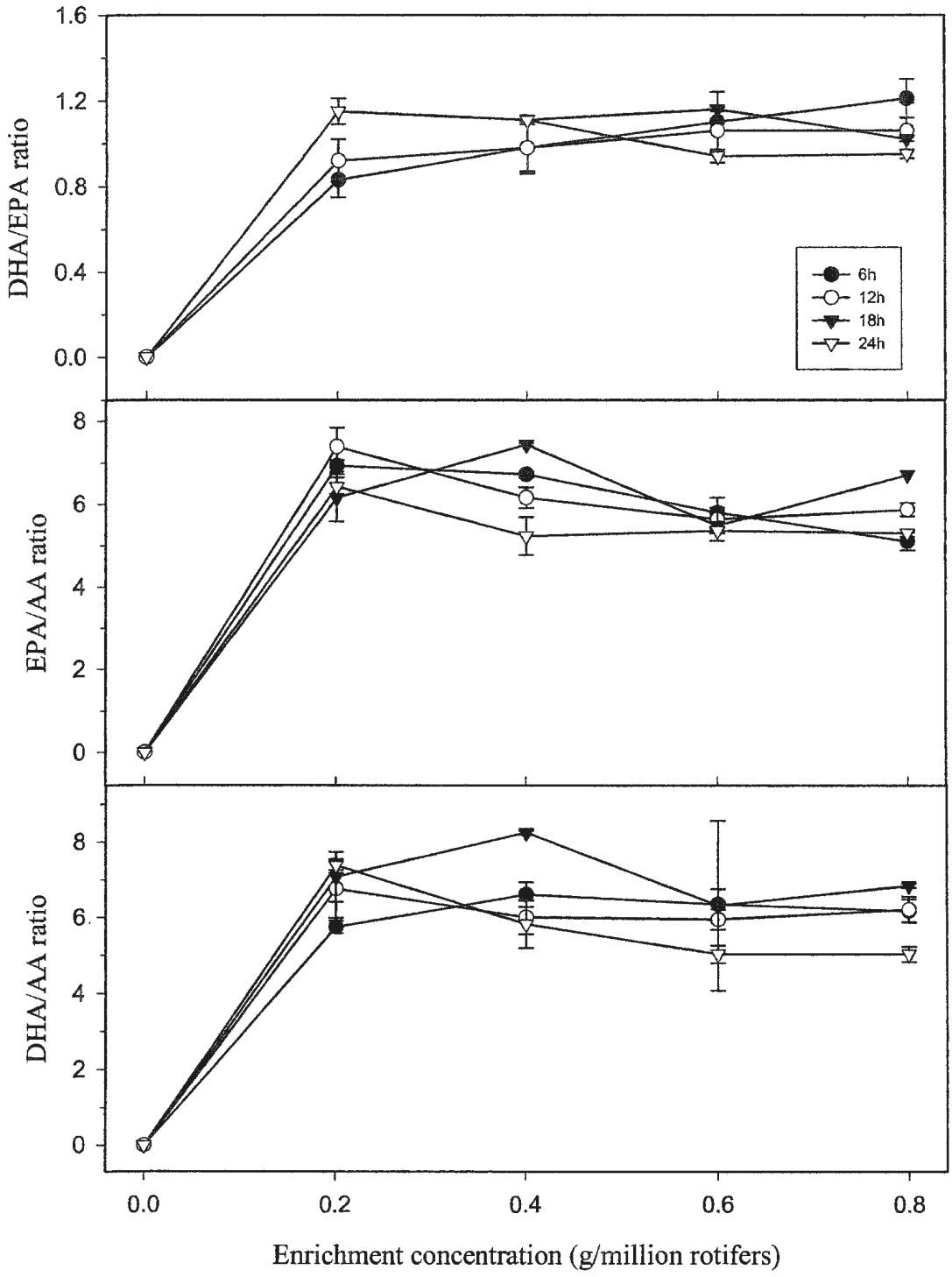

Fig. 5.1. Essential fatty acid (EFA) ratios in total lipids of rotifers (*Brachionus plicatilis*) enriched with seal oil at different concentrations and enrichment periods. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; and AA, arachidonic acid. Vertical bars represent standard deviations, n = 3. For initial values, see Appendix 2.2.

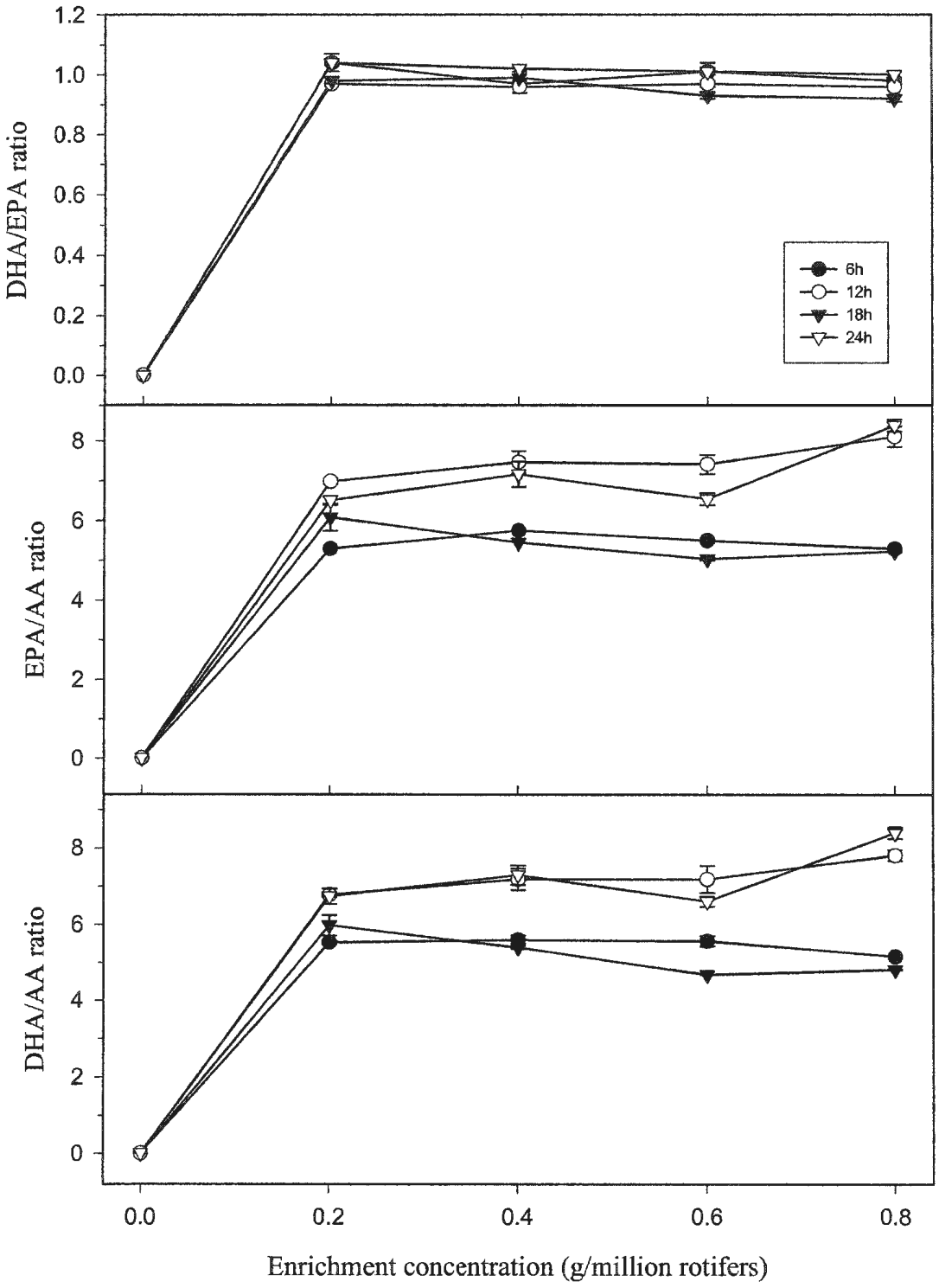

Fig. 5.2. Essential fatty acid (EFA) ratios in total lipids of rotifers (*Brachionus plicatilis*) enriched with menhaden oil at different concentrations and enrichment periods. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid. Vertical bars represent standard deviations, n = 3. For initial values, see Appendix 2.2.

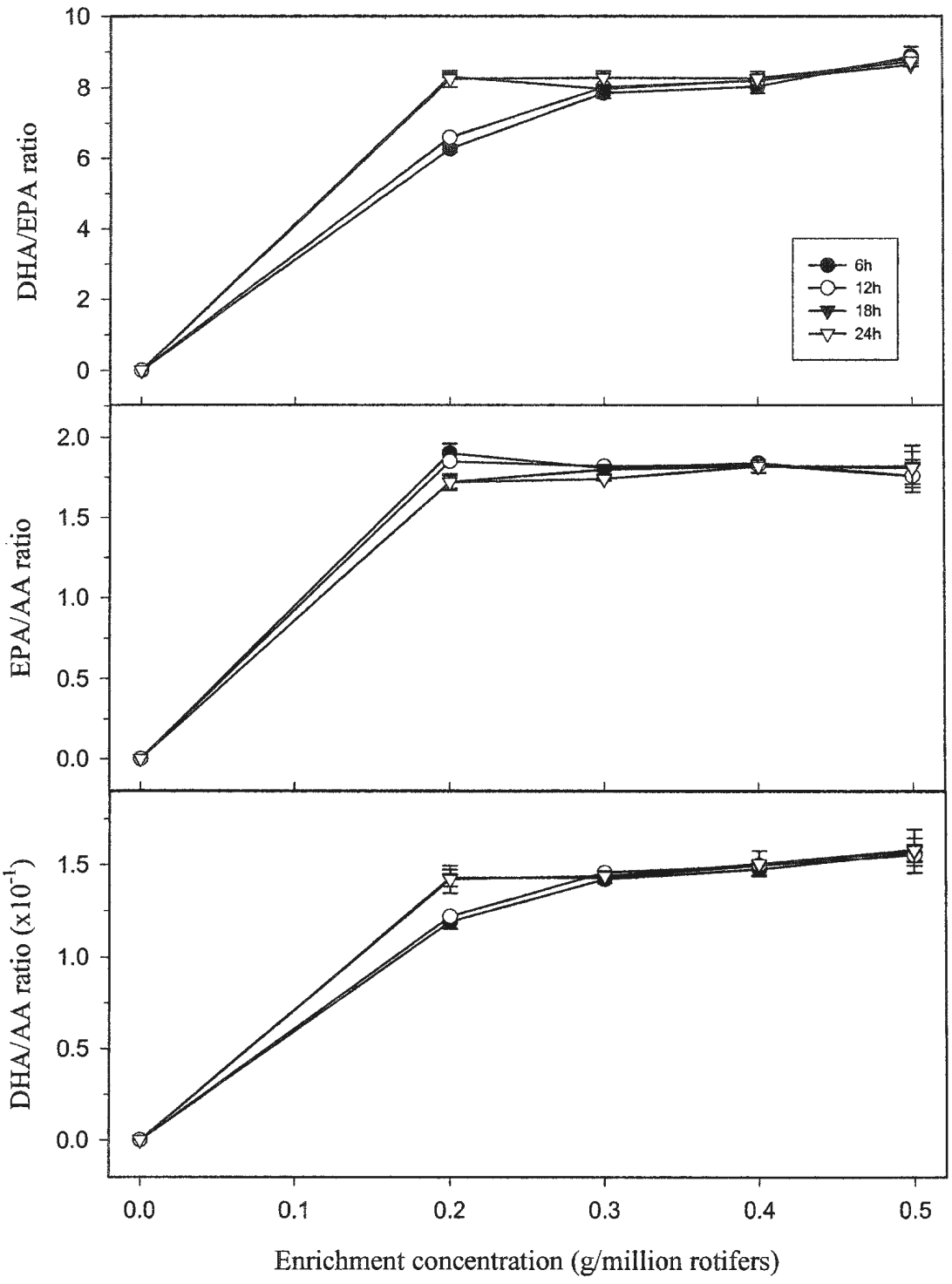

Fig. 5.3. Essential fatty acid (EFA) ratios in total lipids of rotifers (*Brachionus plicatilis*) enriched with Algamac-3010 at different concentrations and enrichment periods. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid. Vertical bars represent standard deviations, n = 3. For initial values, see Appendix 2.2.

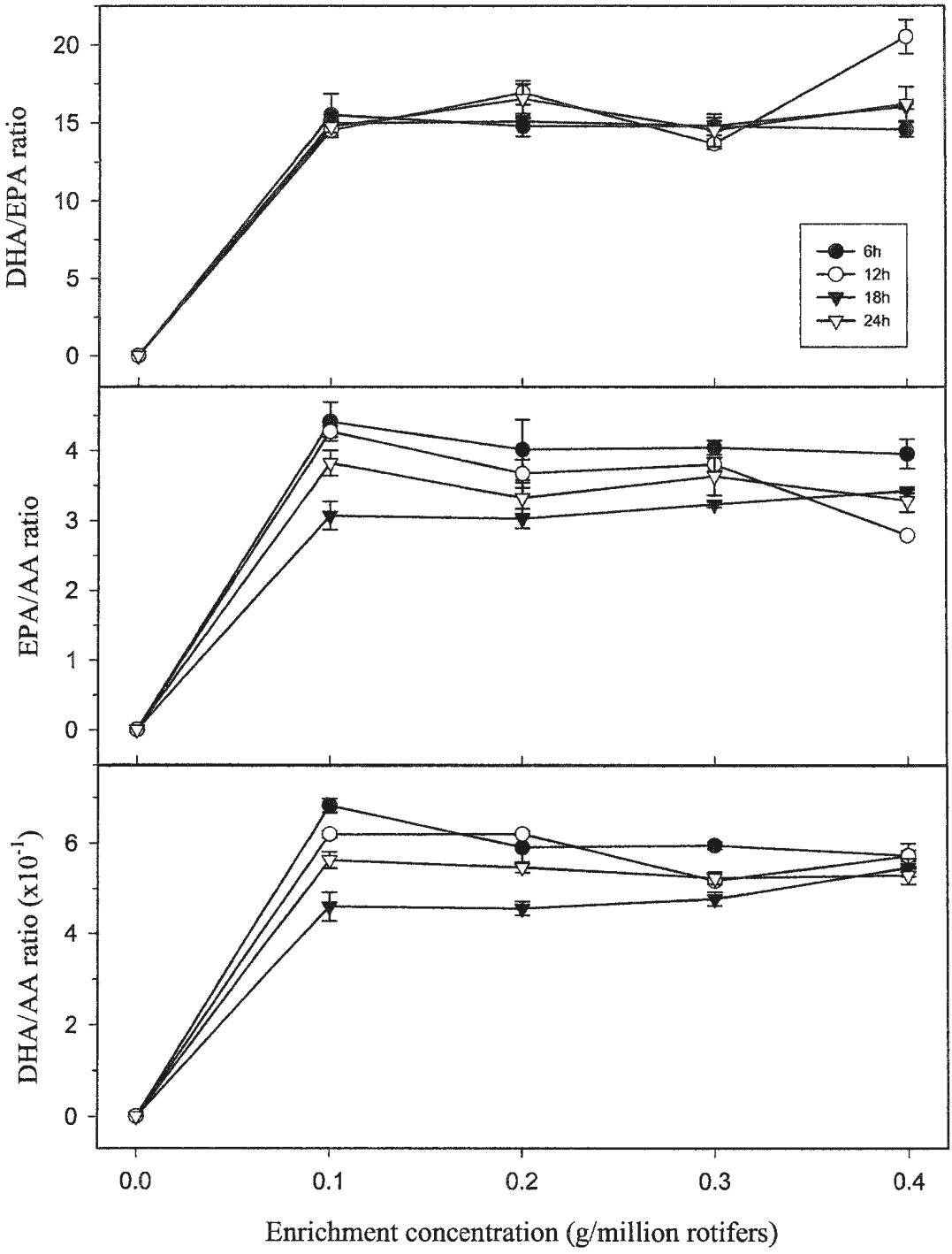

Fig. 5.4. Essential fatty acid (EFA) ratios in total lipids of rotifers (*Brachionus plicatilis*) enriched with DHASCO at different concentrations and enrichment periods. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid; DHASCO, DHA-rich single cell oil. Verical bars represent standard deviations, n = 3. For initial values, see Appendix 2.2.

unaffected by the enrichment concentration. These ratios were, however, higher ($p<0.05$) for the shorter enrichment periods.

5.3.3 Effects of emulsion concentrations and enrichment periods on EFA levels in rotifers

5.3.3.1 Docosahexaenoic acid (DHA)

The effects of emulsion concentration and enrichment period on the contents of DHA in rotifers enriched with different oil emulsions are given in Table 5.3. Two-way ANOVA indicated that the content of DHA in seal oil-, menhaden oil-, Algamac- and DHASCO-enriched rotifers was affected ($p \leq 0.03$) by emulsion concentration and enrichment period (Table 5.2). Similarly, the interaction effect of the enrichment concentration and period on the DHA content was significant ($p < 0.001$) for all treatments, except for Algamac-enriched rotifers ($p = 0.11$). Regression coefficient also indicated a significant correlation between DHA content and the enrichment concentration and period in rotifers fed seal oil ($r = 0.632$; $p < 0.001$), menhaden oil ($r = 0.693$; $p < 0.001$) and Algamac ($r = 0.452$; $p = 0.006$). In DHASCO-fed rotifers, no correlation ($r = 0.336$; $p = 0.07$) between DHA content and the enrichment concentration and period was observed. The DHA content of rotifers enriched with seal oil, menhaden oil, Algamac and DHASCO can be predicted using regression equations: $Y = 4.187 - 0.310C + 0.121P$, $Y = 9.975 - 3.767C - 0.049P$, $Y = 24.64 + 3.133C + 0.021P$ and $Y = 26.42 + 2.697C - 0.095P$, respectively, where Y = DHA content (% total fatty acids), P = enrichment period (h), and C = enrichment concentration (g/million prey).

Table 5.3. Docosahexaenoic acid (DHA) content (% total fatty acids) in rotifers (*Brachionus plicatilis*) enriched with different oil emulsions at different concentrations and enrichment periods.

Enrichment period (h)	Emulsion concentration (g/million rotifers)							
	0.2		0.4		0.6		0.8	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Seal oil								
6	$3.94^{a,x}$	0.15	$4.71^{b,x}$	0.22	$4.50^{ab,x}$	0.30	$4.86^{a,x}$	0.26
12	4.92^{x}	0.33	5.23^{x}	0.29	5.18^{xz}	0.17	5.39^{x}	0.17
18	$7.06^{a,y}$	0.19	$8.27^{b,y}$	0.12	$8.29^{b,y}$	0.22	$6.62^{a,y}$	0.19
24	6.85^{y}	0.64	6.58^{z}	0.56	5.56^{z}	0.43	5.50^{x}	0.42
Menhaden oil								
6	7.34^{x}	0.24	7.42^{x}	0.15	7.48^{x}	0.29	7.52^{x}	0.35
12	$9.27^{a,y}$	0.29	$8.81^{ab,y}$	0.35	$8.24^{bc,y}$	0.41	$7.41^{c,x}$	0.24
18	$8.28^{a,z}$	0.24	$7.21^{b,x}$	0.14	$5.55^{c,z}$	0.13	$4.58^{d,y}$	0.14
24	$8.46^{a,z}$	0.33	$8.53^{a,yz}$	0.27	$6.24^{b,z}$	0.16	$5.28^{c,z}$	0.19
Algamac	0.2		0.3		0.4		0.5	
6	24.59^{a}	0.73	25.41^{ab}	0.51	25.91^{ab}	0.57	26.76^{b}	0.28
12	26.71	1.58	26.25	0.19	26.55	0.13	26.24	1.01
18	25.76	0.27	25.20	0.44	26.16	0.52	25.84	0.23
24	25.57^{a}	0.44	25.85^{ab}	0.41	26.48^{bc}	0.02	26.95^{c}	0.43
DHASCO	0.1		0.2		0.3		0.4	
6	25.38^{a}	0.79	$27.12^{b,x}$	0.25	$28.00^{b,x}$	0.50	$28.38^{b,x}$	0.37
12	25.93^{ab}	0.37	$27.91^{a,x}$	1.63	$24.41^{ab,y}$	0.20	$23.20^{b,y}$	2.18
18	24.16^{a}	0.66	$23.31^{a,y}$	0.44	$21.99^{b,z}$	0.41	$28.46^{c,x}$	0.22
24	25.05^{ab}	1.05	$26.86^{a,x}$	1.53	$24.41^{b,y}$	0.33	$26.20^{a,xy}$	1.17

Analyses were carried out in triplicates. SD, standard deviation. DHASCO, DHA-rich single cell oil.
Values in each row with different superscripts (a,b,c,d) are different ($p<0.05$) from one another.
Values in each column for each emulsion source with different superscripts (w,x,y,z) are different ($p<0.05$) from one another.

5.3.3.2 Eicosapentaenoic acid (EPA)

The contents of EPA in rotifers enriched with various oil emulsions at different enrichment periods are given in Table 5.4. In the seal oil-, menhaden oil- and Algamac-fed rotifers, the content of EPA was affected by both the enrichment concentration and period ($p < 0.001$), but was not affected by the enrichment concentration ($p = 0.20$) in the DHASCO-fed rotifers (Table 5.2). A significant interaction effect ($p < 0.001$) of the enrichment concentration and period, however, existed in all enrichment treatments. Regression analysis indicated the existence of significant correlation between EPA content and the enrichment concentration and period in the seal oil- ($r = 0.717$; $p < 0.001$), menhaden oil- ($r = 0.661$; $p < 0.001$) and Algamac- ($r = 0.668$; $p < 0.001$) enriched rotifers. No correlation ($r = 0.332$; $p = 0.07$) was observed between EPA content of the DHASCO-fed rotifers and the emulsion concentration and enrichment period. The content of EPA in the seal oil-fed rotifers can be estimated from the equations $Y = 4.307 - 0.499C + 0.101P$ and $Y = 10.01 - 3.398C - 0.057P$ for menhaden oil-fed rotifers. For the Algamac- and DHASCO-enriched rotifers, their EPA content can be estimated from the equation $Y = 4.027 - 1.568C - 0.016P$ and $Y = 1.843 - 0.131C - 0.009P$, respectively.

5.3.3.3 Arachidonic acid (AA)

The effect of emulsion concentration and enrichment period on the AA content of rotifers is presented in Table 5.5. Two-way ANOVA showed that the content of AA in rotifers fed seal oil, menhaden oil, Algamac and DHASCO emulsions was affected ($p<0.001$) by

Table 5.4. Eicosapentaenoic acid (EPA) content (% total fatty acids) in rotifers (*Brachionus plicatilis*) enriched with different oil emulsions at different concentrations and enrichment periods.

Enrichment period (h)	Emulsion concentration (g/million rotifers)							
	0.2		0.4		0.6		0.8	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Seal oil								
6	$4.75^{a,x}$	0.16	$4.78^{a,x}$	0.20	$4.12^{b,w}$	0.23	$4.01^{b,w}$	0.24
12	5.38^{y}	0.12	$5.36^{x,z}$	0.17	$4.91^{x,y}$	0.26	5.08^{x}	0.16
18	$6.15^{a,z}$	0.17	$7.44^{b,y}$	0.10	$7.17^{b,y}$	0.12	$6.47^{a,y}$	0.14
24	5.95^{z}	0.26	5.92^{z}	0.39	5.92^{z}	0.26	5.80^{z}	0.29
Menhaden oil								
6	$7.04^{a,x}$	0.18	$7.63^{b,x}$	0.15	$7.40^{ab,x}$	0.18	$7.71^{b,x}$	0.21
12	$9.57^{a,y}$	0.10	$9.16^{a,y}$	0.24	$8.49^{b,y}$	0.30	$7.70^{c,x}$	0.14
18	$8.43^{a,z}$	0.14	$7.28^{b,x}$	0.18	$5.97^{c,z}$	0.18	$4.96^{d,y}$	0.14
24	$8.18^{a,z}$	0.24	$8.38^{a,z}$	0.16	$6.19^{b,z}$	0.09	$5.27^{c,y}$	0.19
Algamac	0.2		0.3		0.4		0.5	
6	$3.92^{a,x}$	0.12	3.24^{a}	0.05	3.23^{b}	0.04	3.01^{c}	0.06
12	$4.05^{a,x}$	0.24	3.28^{b}	0.11	3.24^{b}	0.12	2.98^{b}	0.11
18	$3.10^{a,y}$	0.02	3.17^{b}	0.02	3.19^{b}	0.01	2.99^{c}	0.04
24	3.10^{y}	0.03	3.13	0.02	3.21	0.07	3.09	0.09
DHASCO	0.1		0.2		0.3		0.4	
6	1.64^{a}	0.10	$1.84^{b,x}$	0.10	$1.90^{b,x}$	0.04	$1.95^{b,x}$	0.04
12	1.79^{a}	0.08	$1.71^{a,xy}$	0.07	$1.76^{a,xz}$	0.01	$1.18^{b,y}$	0.03
18	1.61^{a}	0.02	$1.55^{b,y}$	0.03	$1.49^{c,y}$	0.00	$1.78^{d,xz}$	0.01
24	1.70	0.11	1.63^{xy}	0.10	1.69^{z}	0.13	1.63^{z}	0.18

Analyses were carried out in triplicates. SD, standard deviation. DHASCO, DHA-rich single cell oil.
Values in each row with different superscripts (a,b,c,d) are different ($p<0.05$) from one another.
Values in each column for each emulsion source with different superscripts (w,x,y,z) are different ($p<0.05$) from one another.

Table 5.5. Arachidonic acid (AA) content (% total fatty acids) in rotifers (*Brachionus plicatilis*) enriched with different oil emulsions at different concentrations and enrichment periods.

Enrichment period (h)	Emulsion concentration (g/million rotifers)							
	0.2		0.4		0.6		0.8	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Seal oil								
6	0.69^{x}	0.03	0.71^{x}	0.04	0.71^{x}	0.05	0.79^{x}	0.05
12	0.73^{x}	0.04	0.87^{y}	0.08	0.87^{x}	0.09	0.87^{xy}	0.04
18	$1.00^{a,y}$	0.08	$1.00^{a,yz}$	0.00	$1.08^{b,y}$	0.07	$0.97^{a,y}$	0.02
24	$0.93^{a,y}$	0.07	$1.13^{b,z}$	0.05	$1.11^{b,y}$	0.04	$1.09^{b,z}$	0.04
Menhaden oil								
6	1.33	0.09	$1.33^{x,y}$	0.06	1.35^{x}	0.05	1.46^{x}	0.09
12	1.37	0.01	$1.23^{b,xz}$	0.04	$1.15^{c,y}$	0.03	$0.95^{d,y}$	0.01
18	1.39^{a}	0.07	$1.34^{a,y}$	0.02	$1.19^{b,y}$	0.01	$0.95^{c,y}$	0.01
24	1.26^{a}	0.01	$1.17^{b,z}$	0.03	$0.95^{c,z}$	0.01	$0.63^{d,z}$	0.01
Algamac	0.2		0.3		0.4		0.5	
6	$2.07^{a,x}$	0.01	1.79^{b}	0.03	1.76^{b}	0.01	1.71^{b}	0.06
12	$2.19^{a,x}$	0.14	1.80^{b}	0.10	1.77^{b}	0.09	1.69^{b}	0.08
18	1.81^{y}	0.04	1.76	0.01	1.75	0.03	1.65	0.14
24	1.80^{y}	0.06	1.80	0.01	1.76	0.08	1.70	0.04
DHASCO	0.1		0.2		0.3		0.4	
6	$0.37^{a,x}$	0.02	$0.46^{b,x}$	0.02	0.47^{b}	0.00	$0.50^{b,x}$	0.02
12	$0.42^{a,xz}$	0.01	$0.47^{b,xy}$	0.01	0.47^{b}	0.00	$0.42^{a,y}$	0.01
18	$0.53^{a,y}$	0.04	$0.51^{ab,y}$	0.01	0.46^{b}	0.01	$0.52^{a,x}$	0.00
24	0.45^{z}	0.01	0.49^{xy}	0.02	0.47	0.01	0.50^{x}	0.03

Analyses were carried out in triplicates. SD, standard deviation. DHASCO, DHA-rich single cell oil.
Values in each row with different superscripts (a,b,c,d) are different ($p<0.05$) from one another.
Values in each column for each emulsion source with different superscripts (w,x,y,z) are different ($p<0.05$) from one another.

both the enrichment concentration and period. The interaction effect of the enrichment concentration and period was also significant ($p \leq 0.02$) in all feeding treatments (Table 5.2). Similarly, regression analysis indicated a significant relationship between these parameters ($r = 0.892$; $p < 0.001$) and the AA content of seal oil-fed rotifers. A significant correlation between the enrichment concentration and period and the AA content of menhaden oil- ($r = 0.797$; $p < 0.001$), Algamac- ($r = 0.709$; $p < 0.001$) and DHASCO- ($r = 0.480$; $p = 0.003$) fed rotifers was also observed. The content of AA can be estimated using the regression equation $Y = 0.537 + 0.146C + 0.020P$ for seal oil-, $Y = 1.736 - 0.564C - 0.018P$ for menhaden oil-, $Y = 2.185 - 0.868C - 0.005P$ for Algamac- and $Y = 0.406 + 0.112C + 0.002P$ for DHASCO-enriched rotifers.

5.4 Discussion

The rotifer (*Brachionus plicatilis*) enriched with different oil emulsions showed an increase in their total lipid content, although generally insignificant, as the amount of oil in the culture medium and enrichment period increased, similar to the results reported by Rodriguez *et al.* (1996). An increased lipid content of rotifers after enrichment has also been reported by Fernandez-Reiriz *et al.* (1993), Rainuzzo *et al.* (1994), Reitan *et al.* (1994) and Rodriguez *et al.* (1994, 1997). The effect of enrichment concentration and period on the total lipid content was shown by the seal oil-, menhaden oil- and Algamac-enriched rotifers, whereas in DHASCO-fed rotifers, only enrichment period exerted an effect. In seal oil and menhaden oil treatments, an interaction between the enrichment concentration and feeding period exerted a significant effect on the total lipid contents of

the rotifers. The existence of interaction between the enrichment concentration and period indicated that these two parameters modulated against each other in their effect on the lipid content of the rotifers. Within each oil source, differences in the total lipid contents of the prey among enrichment concentration and period were evident. The total lipid content of enriched rotifers in this study (2.85 – 3.89%, wet wt.) was much lower than those reported for Selco products-enriched rotifers (6.1 – 7.1%, wet wt.) by Blair *et al.* (1998). However, these authors did not report the oil concentrations used in their study.

The fatty acids of enriched rotifers, in general term, reflected their corresponding dietary fatty acid profiles, thus, lending further support to previously reported results (Rainuzzo *et al.,* 1989; Rodriguez *et al.,* 1994, 1997). However, certain patterns can be observed in the incorporation of different groups of fatty acids, probably due to their metabolic activities or enzymatic affinities. Fatty acids that were present in high amounts in enrichment media, such as 16:0, 16:1n-7, 18:1n-9 and 22:6n-3 also existed as the dominant fatty acids in enriched rotifers. The first three fatty acids were, however, present at high concentrations in unenriched rotifers, whereas the latter was undetected. The presence of these fatty acids at high levels in enriched rotifers indicates their important roles in the maintenance of the cell membrane integrity. On the other hand, rotifers did not maintain their 18:3n-3 contents as this fatty acid was found at very low levels in enriched prey, despite its presence in high amounts in their unenriched counterparts. Since the contents of 18:3n-3 were very low (0 – 1.61%) in the enrichment diets, it is not clear whether 18:3n-3 in rotifers is dietary dependent. It is clear, however,

that 18:3n-3 was extensively metabolize along with other fatty acids, such as 20:1n-9. Although the content of 20:1n-9 was high (10.2%) in seal oil, its level in enriched rotifers remained much lower compared to its dietary level. Thus, the fatty acid composition of enriched rotifers can be inferred from their dietary fatty acid composition.

In rotifers enriched with seal oil and menhaden oil, the DHA, EPA and AA levels increased with increasing enrichment concentration and period. However, this increase was only evident up to 18 h of enrichment for DHA and EPA after which their levels decreased. On the other hand, AA showed a steady, although not always significant, increase throughout the enrichment periods. The decrease in DHA and EPA contents after 18 h of enrichment period might be due to either the rotifers catabolizing these fatty acids at higher rates than their assimilation rates, or the oil concentrations used were insufficient to support a constant increase as was suggested by Rodriguez *et al.* (1996), or both. It is unlikely that this decrease was due to rotifers losing their gut content as suggested by Dhert *et al.* (2001), since enrichment period of more than 18 h is usually considered as long-term.

Considering the presence of EPA (2.72%) and the absence of DHA in the original rotifers, and that the enriched rotifers maintained their DHA/EPA ratio at approximately 1 when given an emulsion containing an equal amount of DHA and EPA, it is clear that DHA was assimilated better than EPA into rotifers body lipids. This is in contrast to the findings of Rodriguez *et al.* (1996) who reported that EPA was accumulated better than DHA in rotifers, since rotifers enriched with ME maintained the DHA/EPA between 0.66 and 1, despite the fact that ME contained twice as much DHA than EPA. These authors

used TAG and methyl esters (ME) emulsions containing 11.1% DHA and 25.1% EPA, and 47.4% DHA and 28.5% EPA, respectively. Similar to Rodriguez *et al.* (1996), Blair *et al.* (1998) also reported low DHA/EPA ratios (0.64 – 1.10) in rotifers enriched with Selco type (Super, Dry and High DHA Selco) and microfeast enrichment media. Although DHA Selco contained 39.1% DHA and 8.9% EPA, the content of DHA and the DHA/EPA ratio in enriched rotifers were only 11.7% and 1.10, respectively (Blair *et al.,* 1998). In the present study, the levels of DHA and EPA in seal oil, as well as in menhaden oil, were similar in their respective oil source as shown by their DHA/EPA ratios (1.09 for seal oil and 1.06 for menhaden oil). However, when considering the contribution of DHA and EPA in oils and disregarding the initial EPA content in rotifers, it is clear that both DHA and EPA were equally assimilated by the rotifers. This lends further support to the findings of Dhert *et al.* (2001) that rotifers are not selective for the uptake or catabolism of highly unsaturated fatty acids, and therefore, high HUFA levels can be accumulated without problem.

The better incorporation of DHA than EPA was even more definite when rotifers were enriched with emulsions containing high DHA and low EPA levels. In Algamac- and DHASCO-enriched rotifers, DHA levels increased drastically from undetected to 26.8 and 28.5%, respectively, whereas EPA levels increased only about 50% of its initial levels in Algamac- and even decreased in DHASCO-enriched rotifers. It appears that low EPA content in the dietary sources (0.93 and 0.13% in Algamac and DHASCO, respectively), pose no competitive action against DHA, resulting in high assimilation

rates of DHA in rotifers. The DHA/EPA ratios were, however, lower in rotifers than their dietary DHA/EPA ratios.

The level AA of also increased from undetected in the original rotifers to about 1.1% in seal oil- and 1.46% in menhaden oil- and 2.19% in Algamac-, but only up to 0.53% in DHASCO-enriched rotifers. These represent an increase of up to 3, 2 and 4.5 times compared to the dietary levels in seal oil, menhaden oil and Algamac, respectively, indicating that the content of AA in rotifers is dietary independent. It has previously been reported that, unlike DHA and EPA, dietary AA has only little effect on the level of this fatty acid in rotifers (Blair *et al.,* 1998). It seems that rotifers are able to elongate and desaturate shorter chain n-6 fatty acids to AA, since this fatty acid was neither detected in the unenriched rotifers nor in the DHASCO enrichment medium. Although the use of dietary sources is possible, it is likely that rotifers use their body 18 carbon n-6 fatty acids as substrates for AA synthesis. This is evident from the fact that the contents of 18:2n-6 and particularly 18:3n-6 in DHASCO-fed rotifers were lower than those of their unenriched counterparts, despite the presence of 18:2n-6 (0.93%) in DHASCO oil. Fatty acid 18:3n-6 was nearly completely metabolized by DHASCO-enriched rotifers and its content reached 0.14 – 0.27% from 3% in unenriched rotifers.

The results of the present study clearly demonstrate that the fatty acid content of rotifers was enhanced by the enrichment, and this increase was particularly significant in the case of n-3 HUFA and DHA and this paralleled a decrease in 18:3n-3 fatty acids. The ideal enrichment period for rotifers when using seal oil and menhaden oil was 18 and 12 h, respectively, whereas it was 6 h when using enrichment media containing high

DHA for obtaining good results, without increasing the concentration of the oil in the emulsions. This is in line with the results of Watanabe (1993) who suggested an enrichment period of about 6 h, considering the optimum enrichment period of 12 h for rotifers fed on lipid emulsions. It is worth mentioning, however, that the short-term enrichment with oil emulsions results in lipid-encapsulated rotifers, and that the retention time of the nutrients, which are mainly accumulated in the digestive tract of rotifers, is very short and can create problems when rotifers are not immediately eaten by fish larvae (Dhert *et al.*, 2001). Thus, in order to obtain increased DHA content in rotifers, it is more effective to prolong the enrichment period than to elevate the amount of oil in the emulsion.

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Chapter 6

Effect of Different Enrichment Concentration and Period on Lipid Content and Fatty Acid Composition of *Artemia* (*Artemia franciscana*) nauplii

6.1 Introduction

The brine shrimp, *Artemia* spp., are commonly used as a live feed organism in the first feeding of marine fish larvae. The use of *Artemia* as live prey for fish larvae is essential in commercial hatchery operations because no artificial feed formulation is yet available to completely substitute for *Artemia* (Sorgeloos *et al.*, 2001). However, a major drawback in the use of *Artemia* nauplii is the variability in their content of highly unsaturated fatty acids (HUFAs) (Watanabe *et al.,* 1983; Léger *et al.,* 1986; Webster and Lovell, 1990; Sorgeloos *et al.*, 2001). *Artemia* nauplii do not provide adequate nutrition for fish larvae, and there is now good evidence that this drawback arises from a deficiency in the spectrum of essential fatty acids (EFAs) involved (Sorgeloos *et al.*, 2001). Navarro *et al.* (1992) reported that eicosapentaenoic acid (EPA, 20:5n-3) in *Artemia* can be present in widely differing amounts from 0 to 10% of total fatty acids, whereas docosahexaenoic acid (DHA, 22:6n-3) is absent or present only in trace amounts. Due to the low contents of EPA and especially DHA in commercially available *Artemia* strains, it is essential, and a common practice, to enrich these live prey with emulsions of marine oils. These highly unsaturated fatty acids are essential for marine larvae (Watanabe, 1982), and a suboptimal supply may result in reduced viability, growth

and survival during first feeding, as well as incomplete pigmentation of flatfish juvenile (Olsen *et al.*, 2000).

In response to EFAs deficiencies, and taking advantage of the primitive feeding characteristics of *Artemia* nauplii, it is possible to manipulate the nutritional value of HUFAs-deficient *Artemia* prior to offering them to fish larvae (Sorgeloos *et al.*, 2001). Since the nauplii that have molted into the second instar stage (i.e., about 8 h post-hatch) are non-selective particle feeders (Narciso, *et al.,* 1999; Sorgeloos *et al.*, 2001), simple methods have been developed to incorporate different kinds of products into the *Artemia* prior to feeding to larvae. This method of 'bioencapsulation', also called *Artemia* enrichment or boosting, is widely applied in marine fish and crustacean hatcheries for enhancing the nutritional value of *Artemia* with EFAs (Sorgeloos *et al.*, 2001).

Many workers have described methods of increasing n-3 HUFAs levels and DHA/EPA ratios in *Artemia* nauplii and other live prey species by feeding them with algae, microparticles, ω-yeast or marine oil emulsions (Dhert *et al.,* 1990; Navarro *et al.*, 1993; Merchie *et al.*, 1995; Shields and Bell, 1995; Barclay and Zeller, 1996; Olsen *et al.*, 1997, 2000; Blair *et al.*, 1998; Gapasin *et al.*, 1998). The success and convenience of the latter technique has led to commercially produced n-3 HUFAs enrichment emulsions appearing on the market and these are now widely used in marine hatcheries. While many of these techniques have successfully increased the level of n-3 HUFAs in *Artemia*, the ability to increase the DHA/EPA ratio above 1 has remained problematic (Bell, 1998). As such, most of the enrichment techniques described above still fall somewhat short of the 'target' DHA/EPA ratio of 2 in fish tissues, which is also the value found in

the naupliar and copepodite stages of marine copepod (Fraser *et al.,* 1989; Sargent *et al.,* 1997), as well as larval fish (Parrish *et al.,* 1994).

Oil emulsions have been widely used for the enrichment of live feeds in aquaculture (Dhert *et al.,* 1990; Ostrowski and Divakaran, 1990; Clawson and Lovell, 1992; Stottrup and Attramadal, 1992; Navarro *et al.*, 1993). Emulsion-type preparations have a major advantage over dried enrichment diets as they have very high lipid and HUFAs content. Live feeds, such as *Artemia* are able to absorb these important dietary constituents from an aqueous emulsion to which n-3 HUFAs have been added. It is worth mentioning, however, that the enrichment technique has limitations as *Artemia* are selectively catabolizing some of the nutrients such as DHA and phospholipids. Research on the kinetics of DHA catabolism in various *Artemia* strains has shown that DHA catabolism is strain-dependent and could partially be overcome by the use of strains from different geographical areas (Sorgeloos *et al.*, 2001).

Losses of nutrients may take place if the *Artemia* are not fed to fish larvae immediately after enrichment, or if the retention time for the prey in the fish tanks is too long. DHA is especially unstable in *Artemia* (Danielsen *et al.*, 1995; Evjemo *et al.*, 1997; Olsen *et al.*, 1997), and a reduction of 72% has been observed in the nauplii stage during starvation at 12 °C for 24 h after enrichment (Danielsen *et al.*, 1995). While the DHA level in rotifers (*Brachionus plicatilis*) can be kept high by supplying algae rich in DHA (i.e., *Isochrysis galbana*, Tahiti strain) to the fish larvae along with the rotifers (Reitan *et al.*, 1993), this was not the case for short term enriched *Artemia* (Olsen *et al.*, 1997). A stabilization effect of algal addition on DHA content in *Artemia* was only observed at

high concentration after 48 h of incubation period. This algal concentration was, however, far higher than that normally used in hatcheries (Olsen *et al.*, 1997; 2000).

In line with these limitations, refinement of *Artemia*-based feeding techniques can be seen as critical to the establishment of reliable intensive cultivation methods for marine fish. The present experiment was aimed at determining the effects of various concentrations of oil emulsions at different enrichment periods on the total lipids, fatty acid composition and essential fatty acid levels in brine shrimp (*Artemia franciscana*).

6.2 Materials and methods

Decapsulated *Artemia fransiscana* cysts (Premium *Artemia* cysts, Sea Dragon, Utah) were incubated in illuminated, aerated seawater at 26-28°C and 30 ppt salinity for about 24 h for hatching. After hatching, nauplii were separated from empty cysts, washed and placed in filtered, aerated seawater. Newly hatched *Artemia* were divided into 48 batches for enrichment with different types and concentrations of emulsion media. Each batch was given an oil emulsion of either seal blubber oil (AMi Company, St. John's, NF), menhaden oil (Omega Protein, Reedville, VA), Algamac-3010 (Aquafauna Bio-Marine, Inc., Hawthorne, CA) or DHASCO (Martek Bioscience Corp., Columbia, MD). The concentrations of seal oil, menhaden oil and algamac-3010 emulsions were 1, 2, 3 and 4 g/million of nauplii, whereas those of DHASCO were 0.5, 1, 1.5 and 2 g/million of nauplii. Emulsions were prepared by vigorously mixing each enrichment medium with 200 mL seawater and 0.4 g raw egg yolk (as emulsifier) for approximately 3 min. Enrichment with each type of emulsion or concentration was carried out in triplicate.

Enrichment of *Artemia* nauplii was conducted 6 hours post-hatch for 6, 12, 18 and 24 h in 48 4-L capacity of buckets (4 concentrations x 4 periods x 3 replicates). The density of nauplii in enrichment media was 125 nauplii/mL. All emulsions were divided into two portions; the second portion was offered to *Artemia* at 3, 6, 9 and 12 h after the first portion was given, for an enrichment period of 6, 12, 18 and 24 h, respectively. At the end of each enrichment period, enriched nauplii were harvested through a 105-μm-mesh screen, washed with filtered seawater to remove traces of oils, and subsequently washed with filtered freshwater to remove salt. Enriched *Artemia* were then dried with paper towels, transferred to 10 mL glass vials and stored under nitrogen at – 20°C until used for analysis.

Lipid extraction, total lipid determination, fatty acid composition analysis and statistical analysis were carried out following the procedures described previously in Chapter 2.

6.3 Results

6.3.1 Total lipid content of enriched *Artemia*

The total lipid content of *Artemia* (*Artemia franciscana*) nauplii enriched with various oil emulsions at different concentrations and enrichment periods are presented in Table 6.1. In general, the total lipid contents of enriched *Artemia* varied among different emulsion concentrations and enrichment periods. Results of two-way ANOVA indicated that both the emulsion concentration and enrichment period affected ($p \leq 0.001$) the total

Table 6.1. Total lipid content (% wet wt.) of *Artemia* nauplii enriched with different oil emulsions at different concentrations and enrichment periods.

Enrichment period (h)	Emulsion concentration (g/million *Artemia*)							
	1		2		3		4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Initial	2.88 ± 0.14							
Seal oil								
6	$2.81^{a,x}$	0.11	$3.00^{ab,w}$	0.07	$3.16^{bc,x}$	0.16	$3.42^{c,x}$	0.11
12	$3.09^{a,xy}$	0.08	$3.23^{ab,x}$	0.09	$3.44^{bc,x}$	0.12	$3.60^{c,x}$	0.07
18	$3.28^{a,y}$	0.10	$3.50^{ac,y}$	0.06	$3.81^{bd,y}$	0.09	$3.71^{cd,x}$	0.18
24	$4.02^{a,z}$	0.14	$4.03^{a,z}$	0.11	$4.99^{b,z}$	0.09	$4.14^{a,y}$	0.12
Menhaden oil								
6	$2.71^{a,x}$	0.12	$2.61^{a,x}$	0.07	$3.29^{b,x}$	0.10	$3.81^{c,x}$	0.15
12	$3.05^{a,xy}$	0.07	$3.14^{ab,y}$	0.12	$3.34^{bc,x}$	0.04	$3.51^{c,y}$	0.07
18	$3.16^{ab,y}$	0.10	$3.32^{ac,y}$	0.10	$3.03^{b,x}$	0.12	$3.47^{c,y}$	0.12
24	$3.97^{a,z}$	0.22	$4.84^{bc,z}$	0.17	$4.26^{ab,y}$	0.35	$5.03^{c,z}$	0.09
Algamac-3010								
6	$2.50^{a,x}$	0.13	$2.53^{a,w}$	0.11	$2.93^{b,x}$	0.07	$3.27^{c,x}$	0.07
12	$3.46^{a,y}$	0.04	$3.79^{b,x}$	0.06	$3.88^{bc,y}$	0.07	$3.95^{c,y}$	0.04
18	$2.88^{a,x}$	0.29	$3.00^{a,y}$	0.16	$3.52^{b,z}$	0.04	$3.63^{b,z}$	0.09
24	$3.50^{ab,y}$	0.04	$3.38^{a,z}$	0.17	$3.61^{ab,z}$	0.13	$3.72^{b,z}$	0.11
DHASCO	0.5		1.0		1.5		2.0	
6	$3.17^{a,x}$	0.07	$3.68^{b,x}$	0.09	$3.91^{c,x}$	0.04	$4.26^{d,x}$	0.06
12	3.20^{x}	0.03	3.28^{y}	0.10	3.36^{y}	0.14	3.38^{y}	0.11
18	$3.89^{a,y}$	0.08	$4.06^{a,z}$	0.08	$3.94^{a,x}$	0.06	$4.28^{b,x}$	0.07
24	3.11^{x}	0.17	3.23^{y}	0.12	3.40^{y}	0.11	3.46^{y}	0.27

Analysis was carried out in triplicates. SD, standard deviation. DHASCO, DHA-rich single cell oil.
Values in each row for each emulsion with different superscripts (a,b,c,d) are different ($p<0.05$) from one another.
Values in each column for each emulsion with different superscripts (w,x,y,z) are different ($p<0.05$) from one another.

lipid content of *Artemia*, regardless of the emulsion source (Table 6.2). An effect of interaction between the emulsion concentration and enrichment period was also observed ($p < 0.001$) in all feeding treatments.

The seal oil-enriched *Artemia* contained total lipid between 2.81 and 4.99% on a wet weight (ww) basis. The lowest lipid content was found in live prey enriched with the lowest emulsion concentration (1 g/million *Artemia*) and at the shortest enrichment period (6 h), while the highest content was observed in the emulsion concentration of 3 g/million *Artemia* at the longest enrichment period (24 h). The total lipid content of *Artemia* fed for 6 and 12 h increased ($p < 0.05$) as the emulsion concentration increased. *Artemia* under 18 and 24 h enrichment periods showed an increase ($p < 0.05$) in its lipid content as the emulsion concentration increased from 1 to 3 g/million prey, but then decreased ($p < 0.05$) when using an emulsion concentration of 4 g/million prey. On the other hand, the lipid content of *Artemia* increased steadily as the enrichment period increased, regardless of the enrichment concentration. Regression analysis showed a significant relationship ($r = 0.879$; $p < 0.001$) between the total lipid content of *Artemia* and the emulsion concentration and enrichment period. The total lipid content of *Artemia* nauplii enriched with seal oil can be predicted using the following equation: $Y = 2.204 + 0.166C + 0.064P$, where Y = total lipid content (% wet wt.), P = enrichment period (h) and C = emulsion concentration (g/million prey).

The menhaden oil-enriched *Artemia* contained between 2.61 and 5.03% (w/w) of lipid. In general, lipid content increased ($p < 0.05$) as the enrichment concentration and period increased, except for the 18 h enrichment period where *Artemia* under 3 g/million

Table 6.2. Results of two-way ANOVA of the effects of enrichment concentrations and periods on the total lipid and essential fatty acid (EFA) contents of *Artemia* enriched with various oil emulsions.

Concentration and period	Oil emulsion			
	Seal oil	Menhaden oil	Algamac-3010	DHASCO
	Total lipid			
Concentration	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Period	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Interaction	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
	DHA			
Concentration	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Period	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Interaction	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
	EPA			
Concentration	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Period	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Interaction	$p = 0.06$	$p < 0.001$	$p < 0.001$	$p < 0.001$
	AA			
Concentration	$p < 0.001$	$p = 0.58$	$p < 0.001$	$p < 0.001$
Period	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Interaction	$p < 0.001$	$p = 0.01$	$p < 0.001$	$p < 0.001$

DHASCO, DHA-rich single cell oil. DHA, docosahexaenoic acid; EPA, eicosahexaenoic acid; AA, arachidonic acid. Significant difference was considered at 5% of probability level.

prey showed slightly, but significantly ($p < 0.05$), lower lipid content. Multiple linear regression analysis indicated a significant correlation ($r = 0.785$; $p < 0.001$) between the total lipid content and the enrichment concentration and period. The lipid content of *Artemia* enriched with menhaden oil can be predicted using the following regression equation: $Y = 1.922 + 0.220C + 0.071P$.

In the Algamac enrichment, the total lipid content of *Artemia* ranged from 2.50 to 3.95% (wet wt.). The increase in the emulsion concentration resulted in an increase in the total lipid content although this was not always significant ($p > 0.05$). Similarly, increasing the enrichment period did not always result in a significant increase in the lipid content of the prey. Multiple linear regression showed the existence of a significant relationship ($r = 0.651$; $p < 0.00$) between the total lipid content of *Artemia* and the emulsion concentration and enrichment period. The content of lipid can be predicted by the regression equation: $Y = 2.421 + 0.198C + 0.029P$.

For the DHASCO-enriched *Artemia*, the total lipid content was between 3.11 and 4.28% (wet wt.). A few variations ($p < 0.05$) existed in the total lipid content among different emulsion concentrations; differences ($p<0.05$) were, however, generally observed among enrichment periods for a given enrichment concentration. The relationship between total lipid of *Artemia* and the enrichment period and concentration was significant ($r = 0.482$; $p = 0.003$), and the total content can be estimated using the equation: $Y = 3.358 + 0.319C - 0.011P$.

6.3.2 Fatty acid composition of total lipid of *Artemia*

The detailed fatty acid composition of *Artemia* enriched with various oil emulsions at different enrichment concentrations and periods is presented in Appendices 6.1 – 6.16.

6.3.2.1 Saturated fatty acids (SFAs)

The saturated fatty acids (SFAs) of enriched *Artemia* consisted mainly of palmitic acid (16:0), contributing 8.55 – 11.46, 10.56 – 12.39, 9.14 – 13.80 and 9.57 – 10.49% to the total fatty acids of seal oil-, menhaden oil-, Algamac- and DHASCO-fed *Artemia*, respectively. Stearic acid (18:0) was found at much lower levels, ranging from 3.68 to 4.94, 4.70 to 5.72, 4.25 to 6.07 and 3.53 to 4.72% of the total fatty acids, respectively. Other SFAs were found in negligible amounts. Although dietary level of myristic acid (14:0) was high (16.45% in Algamac and 15.45% in DHASCO), this fatty acid contributed a small proportion (0.96 – 2.34 and 1.49 – 3.55% in Algamac and DHASCO-fed prey, respectively) to the *Artemia* lipid fatty acids.

6.3.2.2 Monounsaturated fatty acids (MUFAs)

The MUFAs of seal oil-enriched *Artemia* consisted mainly of oleic (18:1n-9) and palmitoleic (16:1n-7) acids. These fatty acids contributed between 15.24 and 23.34% and 4.24 and 8.99% to the total fatty acids, respectively. The fatty acid 18:1n-7 was also found at relatively high proportions between 5.15 and 6.37% of total fatty acids, whereas 20:1n-9 was present between 2.44 and 4.23%. In menhaden oil-fed *Artemia*, 18:1n-9 and

16:1n-7 were found at approximately similar levels (15.71 – 18.84 and 11.21 – 17.23%, respectively). In Algamac-enriched *Artemia*, 18:1n-9 was found at concentrations between 12.93 and 18.38% to the total fatty acids. Fatty acid 18:1n-7 was found at concentrations ranging from 5.21 to 6.15%. Meanwhile, the DHASCO-enriched *Artemia* contained the highest level of 18:1n-9 (20.62 – 23.82%) of the total fatty acids and its content was relatively unaffected by the enrichment concentration and increased slightly with increasing enrichment period. Fatty acids 18:1n-7 and 16:1n-7 also existed at levels between 4.13 and 5.40%, and 2.90 and 3.75% of the total lipids, respectively. The contents of both fatty acids were essentially unaffected by the enrichment concentration and period, although some significant ($p<0.05$) differences existed in some cases.

6.3.2.3 Polyunsaturated fatty acids (PUFAs)

The polyunsaturated fatty acids (PUFAs) of enriched *Artemia* were composed mainly of the n-3 series of fatty acids. α–linolenic acid (18:3n-3) was the most abundant PUFA present in the enriched prey; its concentration ranged from 17.97 to 27.74, 18.13 to 22.54, 21.61 to 25.63 and 17.89 to 23.79% of the total fatty acids of seal oil-, menhaden oil-, Algamac- and DHASCO-enriched *Artemia*, respectively. The content of 18:3n-3, in general, varied ($p<0.05$) among different enrichment concentrations for each enrichment source. Both EPA (20:5n-3) and DHA (22:6n-3) exhibited much lower concentrations compared to that of 18:3n-3. The content of EPA ranged from 4.18 to 5.85, 6.79 to 9.25, 3.17 to 5.48 and 2.65 to 4.18%, whereas DHA ranged from 1.68 to 2.91, 2.49 to 4.85, 4.54 to 6.67 and 3.91 to 13.79% of the total fatty acids of seal oil-,

menhaden oil-, Algamac- and DHASCO-enriched *Artemia*, respectively. The content of both EPA and DHA generally increased with increasing emulsion concentration and enrichment period; this increase, however, was not always significant ($p>0.05$). Other n-3 fatty acids existed at very low concentrations.

Linoleic acid (18:2n-6) contributed the highest proportion to the n-6 fatty acid family of *Artemia* lipids, followed by 18:4n-6. The former was found at concentrations between 3.97 and 5.34%, whereas the latter was between 2.86 and 4.37% of the total fatty acids of seal oil-enriched *Artemia.* The content of both fatty acids generally varied ($p<0.05$) among treatments. In the menhaden oil enriched *Artemia*, the content of 18:2n-6 was between 3.58 and 6.03% and remained unaffected ($p>0.05$) by the enrichment concentration, but was reduced ($p<0.05$) as the enrichment period increased. The fatty acid 18:4n-6 was also found at concentrations similar to that of 18:2n-6, and its content remained generally unaffected ($p>0.05$) by the emulsion concentration and enrichment period. In Algamac feeding, 18:2n-6 contributed 4.13 – 5.31% to the n-6 PUFAs and was relatively similar among different emulsion concentrations and enrichment periods. A similar pattern was also observed for 18:4n-6, but the content of this fatty acid was slightly lower (3.16 – 4.32%) than that of 18:2n-6. In DHASCO-fed *Artemia,* 18:2n-6 and 18:4n-6 existed at concentrations ranging from 4.30 to 5.31% and 2.36 to 3.53%, respectively. The content of both fatty acids tended to decrease with an increase in emulsion concentration, but was relatively unaffected by enrichment period. Arachidonic acid (AA, 20:4n-6) was found at very low concentrations in enriched *Artemia* under all treatments, ranging from 0.73 to 1.46, 1.06 to 1.36, 0.96 to 2.28 and 0.74 to 0.98% of the total fatty acids in seal oil, menhaden oil, Algamac and DHASCO feeding, respectively.

6.3.2.4 Essential fatty acid (EFA) ratio

Due to a better incorporation rate of EPA than DHA, the DHA/EPA ratio remained low in *Artemia* enriched with seal oil emulsion. The DHA/EPA, EPA/AA and DHA/AA ratios generally increased, but not always significantly, with increasing enrichment concentration; however, no clear trend was observed in the change of these EFA ratios with enrichment period (Fig. 6.1). Similarly, the DHA/EPA ratio in menhaden oil-enriched *Artemia* remained low, despite the increase in oil concentration of the enrichment emulsion. Both DHA/EPA and DHA/AA ratios increased as the enrichment concentration increased from 1 to 3 g/million prey, but then decreased afterwards. On the other hand, the EPA/AA ratio increased only from 1 to 2 g/million and then decreased afterwards (Fig. 6.2). While the DHA/EPA ratio in Algamac-enriched *Artemia* increased, although not always significantly, with increasing emulsion concentration, both EPA/AA and DHA/AA ratios remained nearly unchanged (Fig. 6.3). The DHASCO-enriched *Artemia* consistently showed a higher DHA/EPA ratio compared to the ratios in seal oil, menhaden or Algamac-enriched live prey (Fig. 6.4). While the DHA/EPA and DHA/AA ratios generally increased, although not always significantly, with increasing emulsion concentration, the EPA/AA remained relatively constant. No clear trend was observed in the effect of enrichment period on the EFA ratios of DHASCO-enriched *Artemia*.

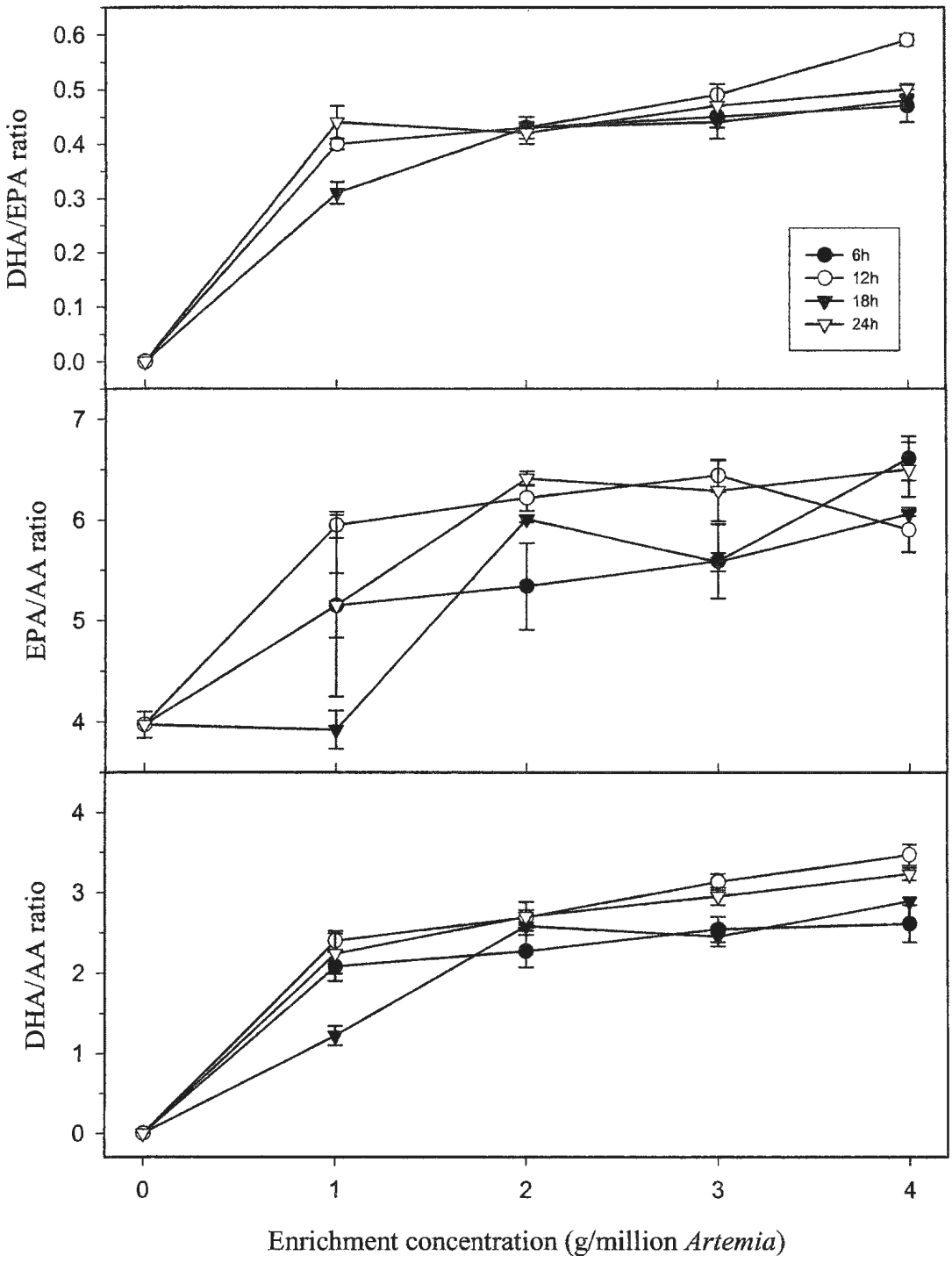

Fig. 6.1. Essential fatty acid (EFA) ratios in total lipids of *Artemia* enriched with seal oil at different concentrations and enrichment periods. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; and AA, arachidonic acid. Vertical bars represent standard deviations, n = 3. For initial values, see Appendix 2.3.

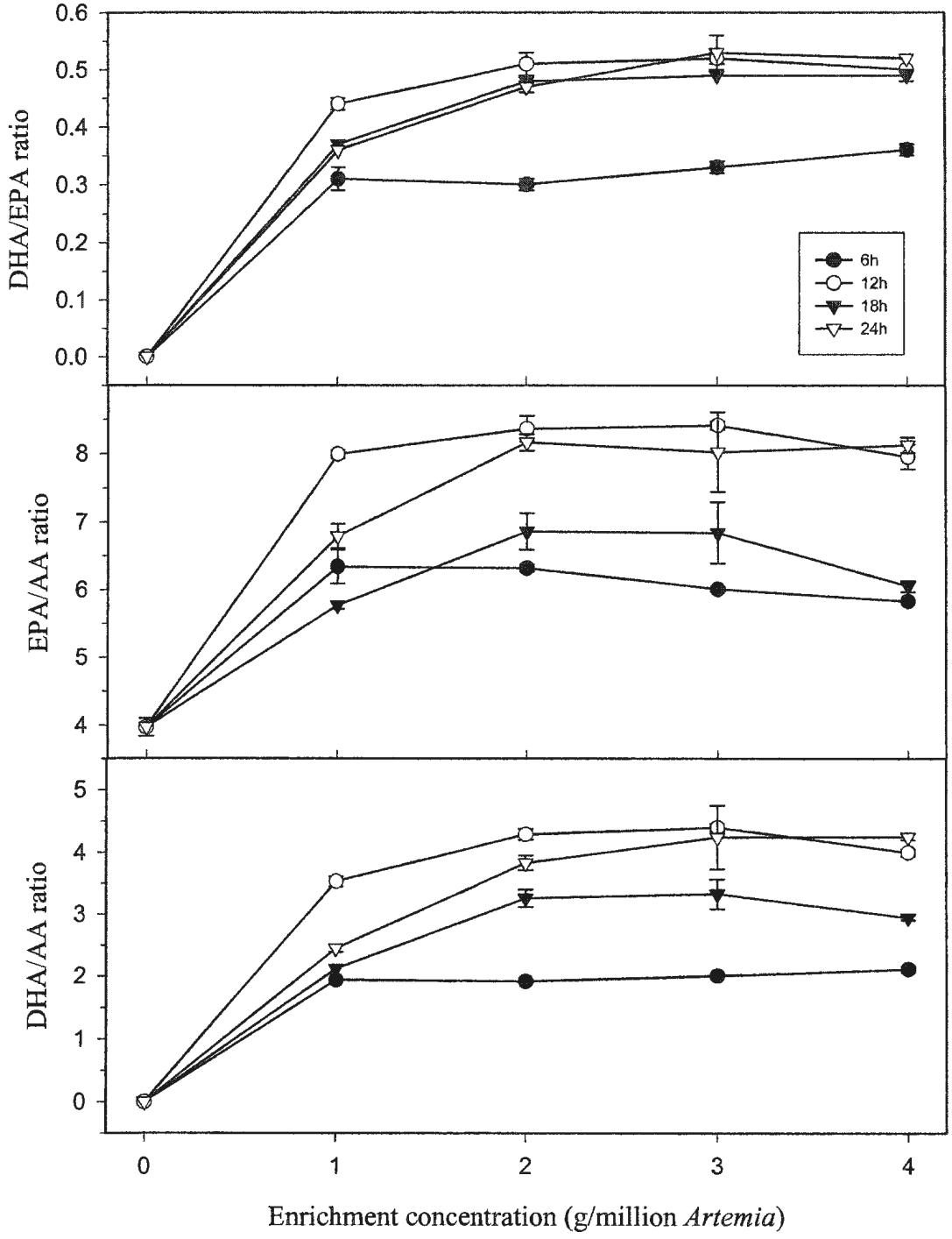

Fig. 6.2. Essential fatty acid (EFA) ratios in total lipids of *Artemia* enriched with menhaden oil at different concentrations and enrichment periods. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid. Vertical bars represent standard deviation, n = 3. For initial values, see Appendix 2.3.

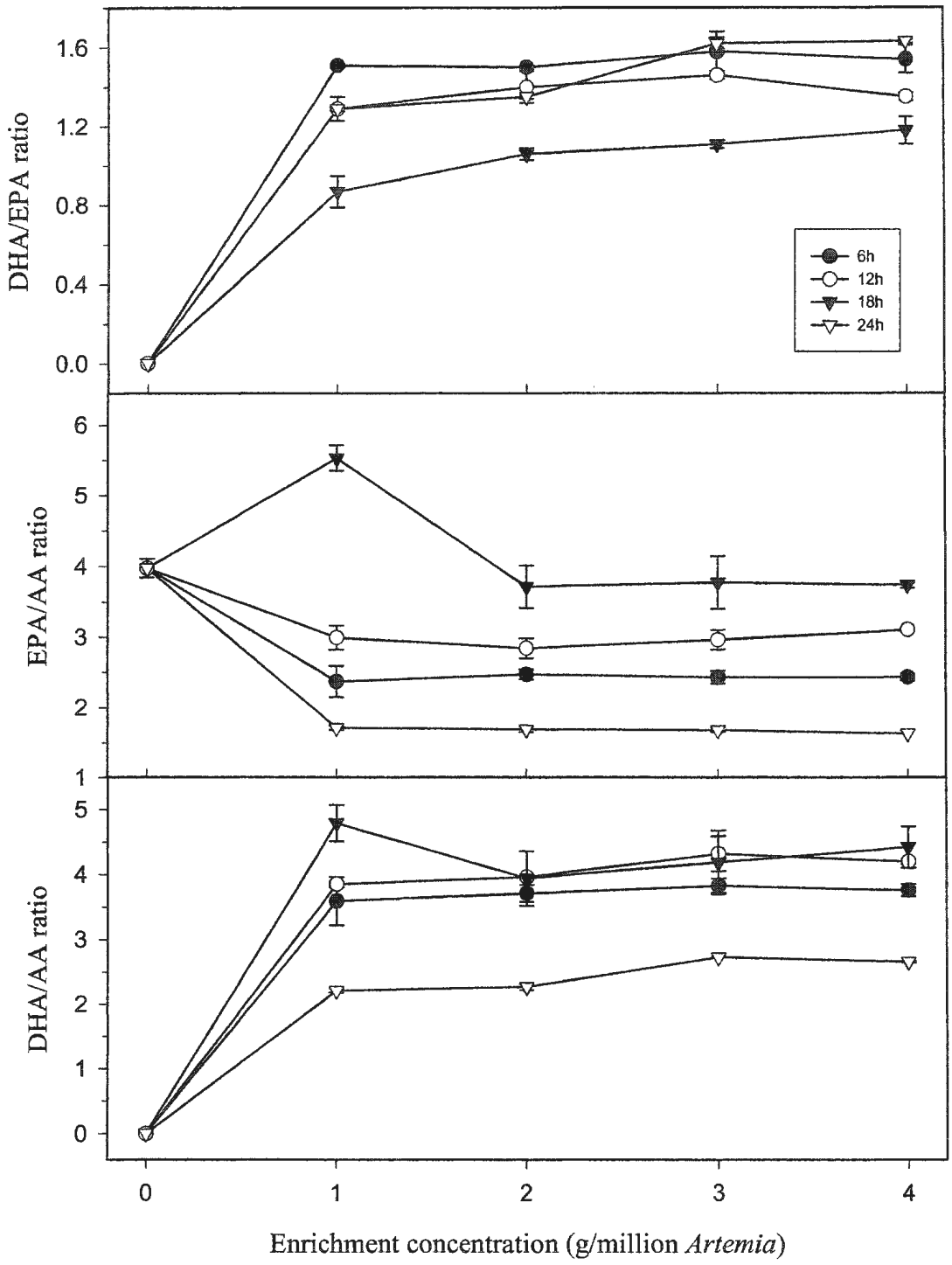

Fig. 6.3. Essential fatty acid (EFA) ratios in total lipids of *Artemia* enriched with Algamac at different concentrations and enrichment periods. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid. Vertical bars represent standard deviation, n = 3. For initial values, see Appendix 2.3.

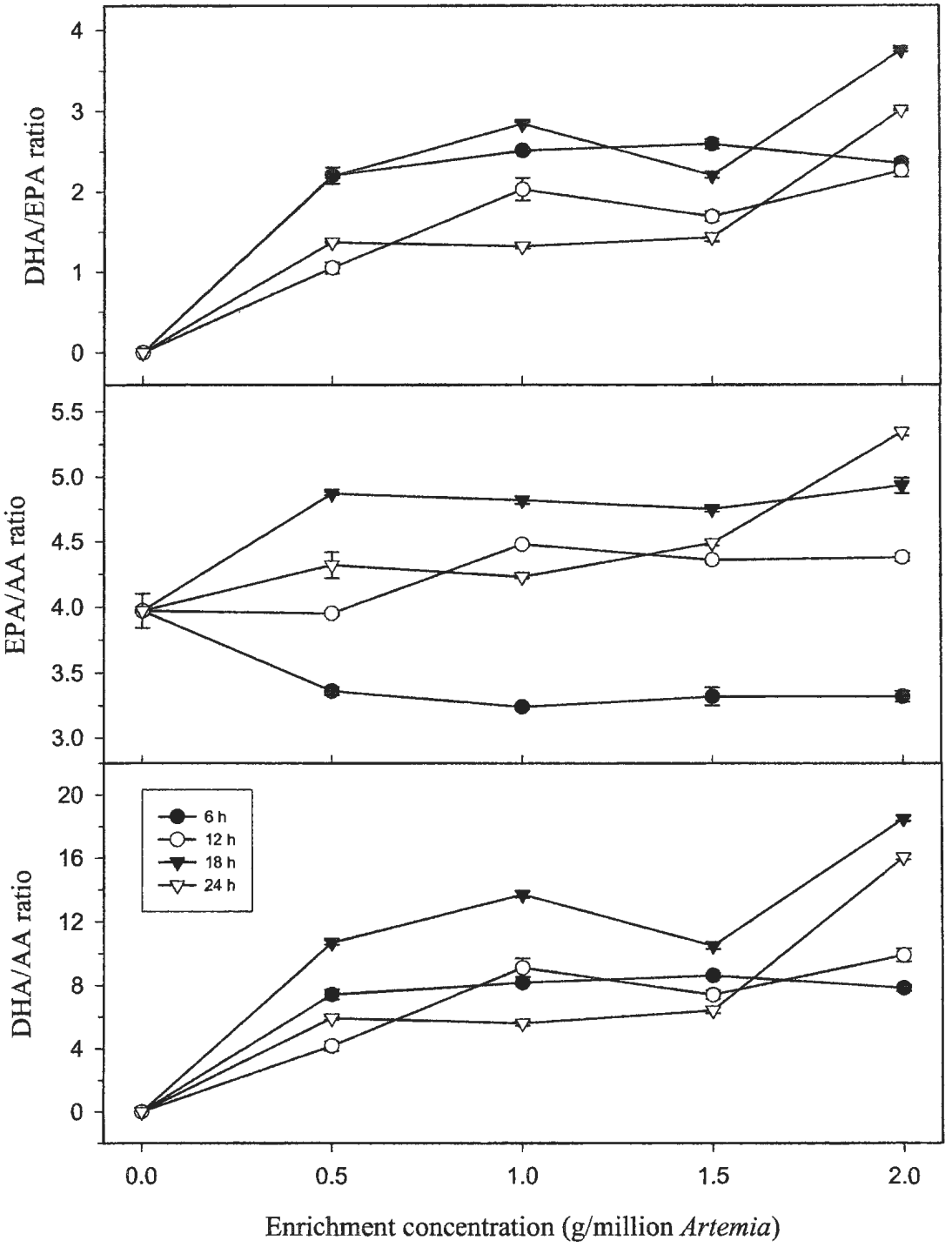

Fig. 6.4. Essential fatty acid (EFA) ratios in total lipids of *Artemia* enriched with DHASCO at different concentrations and enrichment periods. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid. DHASCO, DHA-rich single cell oil. Vertical bars represent standard deviation, n = 3. For initial values, see Appendix 2.3.

6.3.3 Effects of emulsion concentrations and enrichment periods on EFA levels in Artemia

6.3.3.1 Docosahexaenoic acid (DHA)

The contents of DHA in *Artemia* enriched with different oil emulsions at various concentrations and enrichment periods are given in Table 6.3. Two-way ANOVA (Table 6.2) indicated that the content of DHA in seal oil-, menhaden oil-, Algamac and DHASCO-enriched *Artemia* was affected ($p < 0.001$) by both the emulsion concentration and the enrichment period. The interaction between the enrichment concentration and period exerted an effect ($p < 0.001$) on the DHA content of *Artemia*. Regression analysis also indicated a significant correlation ($r = 0.883$; $p < 0.001$) between DHA content of the seal oil-fed *Artemia* and the enrichment concentration and period. A strong relationship between these parameters was also observed in menhaden oil-, Algamac- and DHASCO-enriched *Artemia* ($r = 0.553 - 0.564$, $p < 0.001$). The content of DHA can be predicted using the multiple regression equations: $Y = 1.146 + 0.233C + 0.035P$ for seal oil-, $Y = 2.071 + 0.308C + 0.051P$ for menhaden oil-, $Y = 4.562 + 0.347C + 0.012P$ for Algamac- and $Y = 3.539 + 2.271C + 0.092P$ for DHASCO-enriched *Artemia*, where Y = DHA content (% total fatty acids), P = enrichment period (h), and C = emulsion concentration (g/million prey).

6.3.3.2 Eicosapentaenoic acid (EPA)

As in DHA, the content of EPA in *Artemia* enriched with different oil emulsions was affected ($p < 0.001$) by both the enrichment concentration and period as indicated by the ANOVA (Table 6.4). A significant effect ($p < 0.001$) of the interaction between the

Table 6.3. Docosahexaenoic acid (DHA) content (% total fatty acids) of *Artemia* enriched with different oil emulsions at different concentrations and enrichment periods.

Enrichment period (h)	Emulsion concentration (g/million *Artemia*)							
	1		2		3		4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Seal oil								
6	1.68^{x}	0.13	1.82^{x}	0.15	1.92^{x}	0.11	2.02^{x}	0.18
12	$1.88^{a,x}$	0.12	$2.06^{ab,xz}$	0.12	$2.28^{b,y}$	0.11	$2.91^{c,y}$	0.14
18	$1.78^{a,x}$	0.05	$2.49^{b,y}$	0.03	$2.29^{c,y}$	0.03	$2.74^{d,y}$	0.03
24	$2.27^{a,y}$	0.07	$2.34^{a,yz}$	0.13	$2.65^{b,z}$	0.03	$2.91^{c,y}$	0.03
Menhaden oil								
6	$2.57^{a,x}$	0.07	$2.62^{ab,w}$	0.06	$2.55^{a,x}$	0.08	$2.79^{b,x}$	0.10
12	$3.73^{a,y}$	0.16	$4.65^{b,x}$	0.15	$4.85^{b,y}$	0.15	$4.55^{b,y}$	0.15
18	$2.49^{a,x}$	0.03	$3.70^{b,y}$	0.03	$3.68^{b,z}$	0.11	$3.39^{c,z}$	0.03
24	$2.60^{a,x}$	0.09	$4.29^{b,z}$	0.12	$4.76^{c,y}$	0.13	$4.54^{bc,y}$	0.11
Algamac-3010								
6	4.92^{x}	0.20	5.07^{x}	0.26	5.07^{x}	0.27	4.90^{x}	0.19
12	$5.88^{a,y}$	0.12	$6.19^{ab,y}$	0.21	$6.59^{b,y}$	0.23	$6.67^{b,y}$	0.22
18	$4.61^{a,x}$	0.40	$5.55^{b,xy}$	0.37	$5.91^{bc,z}$	0.05	$6.48^{c,yz}$	0.29
24	$4.54^{a,x}$	0.03	$5.19^{b,x}$	0.04	$6.11^{c,z}$	0.03	$5.97^{d,z}$	0.08
DHASCO	0.5		1		1.5		2	
6	$6.26^{a,w}$	0.25	$6.84^{bc,w}$	0.14	$6.86^{b,x}$	0.15	$6.39^{ac,w}$	0.14
12	$3.91^{a,x}$	0.24	$8.04^{b,x}$	0.50	$6.65^{c,x}$	0.24	$8.49^{b,x}$	0.37
18	$9.05^{a,y}$	0.18	$10.41^{b,y}$	0.11	$8.73^{c,y}$	0.08	$13.79^{d,y}$	0.06
24	$5.41^{a,z}$	0.10	$5.45^{a,z}$	0.14	$5.97^{b,z}$	0.16	$11.94^{c,z}$	0.12

Analyses were carried out in triplicates. SD, standard deviation. DHASCO, DHA-rich single cell oil.
Values in each row with different superscripts (a,b,c,d) are different ($p<0.05$) from one another.
Values in each column for each emulsion source with different superscripts (w,x,y,z) are different ($p<0.05$) from one another.

Table 6.4. Eicosapentaenoic acid (EPA) content (% total fatty acids) of *Artemia* enriched with different oil emulsions at different concentrations and enrichment periods.

Enrichment period (h)	Emulsion concentration (g/million *Artemia*)							
	1		2		3		4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Seal oil								
6	4.18^{x}	0.36	4.28^{x}	0.24	4.22^{x}	0.16	4.35^{x}	0.27
12	$4.67^{x,z}$	0.14	4.78^{y}	0.12	4.69^{xy}	0.15	4.96^{y}	0.12
18	$5.71^{a,y}$	0.13	$5.81^{a,z}$	0.05	$5.21^{b,yz}$	0.10	$5.73^{a,z}$	0.04
24	$5.20^{y,z}$	0.17	5.56^{z}	0.20	5.65^{z}	0.41	5.85^{z}	0.16
Menhaden oil								
6	$8.42^{a,x}$	0.14	$8.62^{a,x}$	0.16	$7.63^{b,x}$	0.17	$7.67^{b,x}$	0.21
12	$8.45^{a,x}$	0.11	$9.06^{ab,y}$	0.10	$9.25^{b,y}$	0.29	$9.03^{ab,y}$	0.36
18	$6.79^{a,y}$	0.13	$7.78^{b,z}$	0.13	$7.55^{b,x}$	0.19	$6.96^{a,z}$	0.17
24	$7.22^{a,z}$	0.17	$9.14^{b,y}$	0.11	$9.01^{bc,y}$	0.20	$8.67^{c,y}$	0.18
Algamac-3010								
6	3.25^{w}	0.10	3.38^{w}	0.14	3.20^{w}	0.04	3.17^{w}	0.01
12	$4.57^{a,x}$	0.11	$4.44^{a,x}$	0.11	$4.25^{a,x}$	0.08	$4.92^{b,x}$	0.09
18	5.31^{y}	0.02	5.22^{y}	0.21	5.32^{y}	0.06	5.48^{y}	0.09
24	$3.52^{a,z}$	0.01	$3.85^{b,z}$	0.04	$3.77^{b,z}$	0.06	$3.66^{c,z}$	0.03
DHASCO	0.5		1		1.5		2	
6	$2.84^{a,w}$	0.01	$2.72^{b,w}$	0.00	$2.65^{c,x}$	0.02	$2.72^{b,x}$	0.00
12	$3.72^{a,x}$	0.01	$3.97^{b,x}$	0.02	$3.93^{b,y}$	0.05	$3.76^{a,y}$	0.04
18	$4.13^{a,y}$	0.04	$3.66^{b,y}$	0.07	$3.97^{a,y}$	0.08	$3.68^{b,y}$	0.06
24	$3.95^{a,z}$	0.05	$4.13^{b,z}$	0.06	$4.18^{b,z}$	0.03	$3.97^{a,z}$	0.03

Analysis was carried out in triplicates. Standard deviation (SD) values <0.01 are reported as 0.00. DHASCO, DHA-rich single cell oil.

Values in each row with different superscripts (a,b,c,d) are different ($p<0.05$) from one another.

Values in each column for each emulsion source with different superscripts (w,x,y,z) are different ($p<0.05$) from one another.

enrichment concentration and period on the EPA content was observed in all feeding treatments, except in the seal oil fed *Artemia* ($p = 0.06$). Regression analysis, however, showed significant correlation between EPA content and the emulsion concentration and enrichment period only in seal oil- ($r = 0.867$; $p < 0.001$) and DHASCO- ($r = 0.832$; $p < 0.001$) enriched *Artemia*. The relationship between these parameters was absent in menhaden oil- and Algamac-enriched *Artemia* ($r = 0.122$, $p = 0.72$ and $r = 0.297$, $p = 0.13$, respectively). The EPA content can be estimated from the following equations: $Y = 3.698 + 0.068C + 0.079P$ for seal oil- and $Y = 2.706 - 0.064C + 0.067P$ for DHASCO-enriched *Artemia*. For menhaden oil- and Algamac-enriched *Artemia*, their EPA contents can be estimated using equations: $Y = 8.119 + 0.077C - 0.007P$ and $Y = 3.586 + 0.035C + 0.036P$, respectively.

6.3.3.3 Arachidonic acid (AA)

The AA contents of *Artemia* enriched with different oil emulsions at various concentrations and enrichment periods are given in (Table 6.5). Results of two-way ANOVA (Table 6.2) showed that the content of this fatty acid in *Artemia* enriched with seal oil, Algamac and DHASCO emulsions was affected ($p < 0.001$) by both the enrichment concentration and period, whereas that of menhaden oil-fed *Artemia* was affected ($p < 0.001$) only by the enrichment period. However, the interaction effect of the enrichment concentration and period on the AA content existed ($p \leq 0.01$) in all feeding treatments. Regression analysis indicated a significant relationship among parameters employed ($r = 0.490 - 0.709$, $p \leq 0.002$) in enriched *Artemia*.

Table 6.5. Arachidonic acid (AA) content (% total fatty acids) of *Artemia* enriched with different oil emulsions at different concentrations and enrichment periods.

Enrichment period (h)	Emulsion concentration (g/million *Artemia*)							
	1		2		3		4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Seal oil								
6	0.81^{x}	0.13	0.80^{xy}	0.12	0.75	0.15	0.78	0.13
12	0.78^{x}	0.02	0.77^{x}	0.06	0.73	0.05	0.84	0.07
18	$1.46^{a,y}$	0.11	$0.97^{b,y}$	0.00	0.93^{b}	0.03	0.95^{b}	0.01
24	1.02^{x}	0.15	0.87^{xy}	0.01	0.90	0.02	0.90	0.01
Menhaden oil								
6	$1.33^{ab,x}$	0.03	$1.36^{a,x}$	0.02	$1.27^{b,x}$	0.03	$1.31^{ab,x}$	0.01
12	$1.06^{a,y}$	0.02	$1.08^{a,y}$	0.01	$1.10^{ab,y}$	0.02	$1.14^{b,y}$	0.03
18	1.18^{z}	0.02	1.13^{y}	0.04	1.11^{xy}	0.05	1.15^{y}	0.00
24	1.06^{y}	0.04	1.12^{y}	0.00	1.13^{xy}	0.11	1.07^{z}	0.04
Algamac-3010								
6	1.37^{w}	0.08	1.37^{x}	0.02	1.32^{x}	0.03	1.30^{w}	0.02
12	1.53^{x}	0.05	1.56^{y}	0.04	1.53^{y}	0.04	1.59^{x}	0.04
18	$0.96^{a,y}$	0.03	$1.41^{b,x}$	0.06	$1.42^{b,xy}$	0.08	$1.47^{b,y}$	0.04
24	$2.06^{a,z}$	0.04	$2.28^{b,z}$	0.06	$2.24^{b,z}$	0.08	$2.24^{b,z}$	0.02
DHASCO	0.5		1		1.5		2	
6	$0.85^{a,x}$	0.00	$0.84^{a,w}$	0.00	$0.80^{b,w}$	0.01	$0.82^{c,x}$	0.03
12	$0.94^{a,y}$	0.01	$0.88^{b,x}$	0.00	$0.90^{c,x}$	0.00	$0.86^{d,x}$	0.00
18	$0.85^{a,x}$	0.01	$0.76^{b,y}$	0.00	$0.84^{a,y}$	0.01	$0.75^{b,y}$	0.01
24	$0.91^{a,y}$	0.03	$0.98^{b,z}$	0.00	$0.93^{a,z}$	0.00	$0.74^{c,y}$	0.01

Analysis was carried out in triplicates. Standard deviation (SD) values <0.01 are reported as 0.00. DHASCO, DHA-rich single cell oil.

Values in each row with different superscripts (a,b,c,d) are different ($p<0.05$) from one another.

Values in each column for each emulsion source with different superscripts (w,x,y,z) are different ($p<0.05$) from one another.

Multiple linear regression provides equations to predict the content of AA, as follow: $Y = 0.847 - 0.050C + 0.011P$ for seal oil- ; $Y = 1.315 + 0.001C - 0.010P$ for menhaden oil- ; $Y = 0.893 + 0.048C + 0.039P$ for Algamac- ; and $Y = 0.901 - 0.057C + 0.002P$ for DHASCO-enriched *Artemia*.

6.4 Discussion

This study examined the influence of varying enrichment concentration and period on lipid and fatty acid composition of *Artemia franciscana* enriched with various oil emulsions. The results showed that, in general, both the enrichment concentration and period affected the total lipid content of enriched *Artemia*. The total lipid content of *Artemia* increased with oil emulsion concentration and enrichment period. An increase of total lipid during enrichment of *Artemia* has been reported to be due to the expansion of the triacylglycerol (TAG) reserves (Takeuchi *et al.,* 1992; McEvoy *et al.,* 1996). The increase in total lipid levels, however, was not always significant. In DHASCO-enriched *Artemia*, there was a decrease in the total lipid content for all enrichment concentrations from 18 to 24 h periods. The higher lipid content in *Artemia* enriched with seal oil and menhaden oil compared to Algamac- or DHASCO-enriched *Artemia* does not necessarily indicate a higher incorporation rate of lipid, but due to higher seal oil and menhaden oil concentration in the emulsions. In all feeding trials, both the oil concentration and the enrichment period significantly affected the total lipid content of *Artemia*. The total lipid content of enriched *Artemia* in the present study were comparable to those reported

previously by Takeuchi *et al.* (1992), McEvoy *et al.* (1996) and Coutteau and Mourente (1997).

The fatty acid composition of the enriched *Artemia* reflected, in general, their dietary composition. It is important to note, however, that *Artemia*, while maintaining their fatty acid species in a relatively narrow range, also intensively utilized fatty acids that were available in high levels in the diets. For example, regardless of the enrichment concentration and period, fatty acids 16:1n-7 and 20:1n-9 were maintained at around 7 – 9 and 3 – 4% respectively, despite their high dietary levels in seal oil (18.5 and 10.2%, respectively). Similarly, 16:0 was maintained in *Artemia* at levels between 8 and 14%, even when the dietary levels of this fatty acid were high (19 – 40%). *Artemia* also maintained 18:3n-3 at levels between 17 and 25%, despite the dietary level of this fatty acid which was present in only 0.18 – 1.61%, and even undetected in DHASCO enrichment medium.

This study showed clearly that *Artemia* accumulated EPA better than DHA, thus, lending further support to the previous findings (Watanabe, 1993; McEvoy *et al.* 1995; Furuita et al., 1996; Navarro *et al.,* 1999; Sargent *et al.,* 1999; Han *et al.,* 2001). Despite the similar levels between EPA and DHA in seal oil and menhaden oil enrichment media, the contents of EPA in *Artemia* enriched with these oils were two to three times higher than that of DHA, resulting in a low DHA/EPA ratio (Append. 6.1-6.8). In the case of very high dietary DHA levels, although the DHA content in Algamac and DHASCO emulsions was 29 and 322 times higher than that of EPA (Chapter 2, Table 2.1), respectively, the contents of DHA in the enriched *Artemia* were only 1.1 – 3.7 times higher than EPA contents (Append. 6.9 – 6.16). It has been reported that DHA

incorporation was always accompanied by an EPA increase, indicating some metabolic conversion of DHA to EPA (McEvoy and Sargent, 1998), or competitive interaction of EPA or AA on DHA incorporation (Han *et al.*, 2001). These latter authors also found that, during subsequent starvation, the presence of DHA in naupliar lipids increased the EPA retention, which might be related to DHA retroconversion. The incorporation rate of DHA seems to be suppressed not only by the presence of high levels of EPA in the enrichment media, but also the presence of EPA and particularly high level of α–linolenic acid (18:3n-3) in the newly hatched *Artemia*. Newly hatched *Artemia franciscana* contained a low level of EPA (2.82%) and a very high level of 18:3n-3 (30%) and oleic acid (18:1n-9, 19.2%). On the other hand, DHA was not detected in this unenriched live prey organism.

It is interesting that *Artemia* enriched with seal and menhaden oils continued, in general, to accumulate DHA up to 24 h of enrichment period, whereas *Artemia* enriched with Algamac and DHASCO accumulated DHA up to 18 h of enrichment period, after which its DHA content decreased. These patterns indicate that, when *Artemia* are enriched with emulsions containing a lower DHA level and that as long as they are kept in the enrichment media, these prey organisms will, to a certain extent, continue to assimilate, or at least maintain their DHA levels in the body lipids. On the other hand, when using emulsions containing high levels of DHA, the level of this fatty acid increases only during relatively short enrichment periods. The tendency for the content of DHA to decrease during long enrichment period, for example 24 h, might have been the result of the higher rates of DHA catabolism than its assimilation rates. Watanabe (1993) suggested an optimum enrichment period of 12 h for *Artemia* fed on lipid

emulsions. McEvoy *et al.* (1995) showed that enrichment periods of 24 h increased the risk of peroxidtion of PUFAs in *Artemia* enrichment medium. These authors suggested that potentially toxic oxidation products could negatively affect the live prey population and possibly the performance of larval fish preying on them. In addition, minimizing the time required for enrichment reduces the cost associated with live feed production (Southgate and Lou, 1995).

The use of DHA concentrates such as DHA Selco, Protein Selco and DHASCO, are commonly used to obtain a higher concentration of n-3 HUFA and DHA/EPA ratio than is possible with TAG fish oils. The much higher DHA levels and DHA/EPA ratios in *Artemia* enriched with DHASCO oil emulsion compared to those in *Artemia* enriched with seal oil, menhaden oil and Algamac indicated that DHA in this oil concentrate was incorporated more effectively. This might, however, be due to the absence of competitive action of EPA and AA since DHASCO oil was devoid from these fatty acids. Nevertheless, the interference from the simultaneous variation of total n-3 HUFA content and DHA/EPA ratio in the emulsions could not be excluded, as has been suggested by Coutteau and Mourente (1997). For example, while the DHA/EPA ratios of seal oil-enriched *Artemia* were similar to those of menhaden oil-enriched prey, the n-3 HUFA levels in the former were lower due to the lower DHA and EPA contents. In contrast, the DHA/EPA ratios in menhaden oil-enriched *Artemia* were much lower than those of their Algamac-enriched counterparts although they showed similar n-3 HUFA levels because of the much higher DHA and lower EPA contents in Algamac enriched *Artemia*. On the other hand, both the n-3 HUFA contents and DHA/EPA ratios in DHASCO-enriched *Artemia* were much higher than those in prey enriched with the other enrichment media.

This was, however, due to the higher DHA and lower EPA contents in the DHASCO-enriched *Artemia*. Nevertheless, the present study also demonstrated that, using Algamac and DHASCO emulsions, it was possible to obtain DHA/EPA ratios similar or even higher than DHA/EPA ratios of 1.5 to 2 in wild zooplankton previously reported by Fraser *et al.* (1989), Lokman (1993), Næss *et al.* (1995) and Sargent *et al.* (1997), despite the reported specific breakdown of DHA in brine shrimp, particularly *Artemia franciscana*, during enrichment (Dhert *et al.,* 1993).

Considering the n-3 HUFA and DHA/EPA ratio of the enrichment media used in the present study, it is clear that the levels of n-3 HUFA and DHA, as well as the DHA/EPA ratio in enriched *Artemia*, were not proportional to their dietary levels or ratios. This is in contrast to the findings of Lemn and Lemarie (1991) and Støttrup and Attramadal (1992) who reported that the HUFA content of enriched *Artemia* was proportional to the HUFA content of the enriching diet. However, this study also showed that the HUFA content of enrichment diets is a major factor in determining the HUFA level of enriched live prey, as reported previously (Lemn and Lemarie, 1991; Støttrup and Attramadal, 1992).

The concentration of AA in *Artemia* was independent of its dietary levels. This was evident from the fact that *Artemia* enriched with Algamac contained consistently higher levels of AA compared to those of *Artemia* enriched with seal oil and menhaden oil, despite the fact that the Algamac medium contained AA at a level similar to that of seal oil and almost twice as low as that of menhaden oil. Also, *Artemia* appears to strictly control the level of AA, suggesting its physiological significance. For example, although DHASCO is devoid of this fatty acid, DHASCO-fed *Artemia* maintained their AA at

levels comparable to those in their seal oil-fed counterparts. These results confirmed the findings of Zhukova *et al.* (1998).

In the present experiments, it was possible to reach high levels of n-3 HUFA, but nevertheless, the DHA/EPA ratios in seal oil, menhaden oil and Algamac-enriched *Artemia* were still much lower than the proposed ratio of ≥ 2 (Sargent, 1995; Narciso *et al.*, 1999). A high DHA/EPA ratio (>2) can only be obtained by using an oil emulsion containing a very high level of DHA and a negligible amount of EPA. *Artemia* are known to accumulate DHA with difficulty even when the diet is rich in this fatty acid. McEvoy *et al.* (1995) have shown that, in *Artemia* nauplii enriched with different fish oils, the final DHA/EPA ratio was invariably lower than that in the original media, suggesting that the ratio could be altered metabolically by the *Artemia* themselves (Dhert *et al.,* 1993). In the present study, the final DHA/EPA ratios in *Artemia* ranged from 0.30 to 3.75 although the dietary ratios varied from 1.06 to 321. A possible explanation for the lower accumulation of DHA than EPA might be a faster autoxidation rate of DHA in the enrichment medium. However, examination of the DHA/EPA ratio of the *Artemia* medium over time negated this hypothesis (McEvoy *et al.,* 1995). Instead, it appears that *Artemia* might metabolize DHA to a larger extent than EPA. Therefore, the assimilation capacities of live prey for both fatty acids should be taken into account when using them for larval feeding (Rodriguez *et al.,* 1996).

The present study indicated that both the enrichment concentration and period as well as the interaction between them significantly affected the total lipid, DHA, EPA and AA contents of *Artemia*. However, in order to obtain increased EFAs, particularly DHA and AA, as well as the n-3 HUFA contents in *Artemia*, it is more effective to prolong the

enrichment period than to increase the oil concentration in the enrichment emulsion. It is worth mentioning, however, that enrichment studies, as have been suggested by Coutteau and Mourente (1997), only evaluate the end result of a chain of biological processes, including ingestion, digestion and metabolic conversion. As each of these processes may be influenced in different ways by the choice of the n-3 HUFA source, it is not possible to conclude on the relative digestibility of each of the oils used in the present study. In addition, although no physical appearance differences were observed among oil-based emulsions used in this study, it is not known to what extent different oil sources may cause small shifts in particle size distribution and/or stability of emulsions which may in turn affect the filtering efficiency of *Artemia*.

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Chapter 7

Summary and Recommendations

7.1 Summary

The effect of enrichment on the fatty acid profiles of live feeds and its subsequent effect on yellowtail flounder (*Limanda ferruginea*) larvae was examined. Live feeds used in the first experiment were enriched with seal oil, menhaden oil and Algamac, and with seal oil, seal oil+DHASCO and Algamac in the second experiment. The survival rates of flounder larvae ranged from 11.1 to 20.4 % in the first experiment and from 5.7 to 9.9% in the second experiment, being highest in seal oil- and lowest in Algamac-fed fish in both set-ups. However, a significant difference existed only between seal oil- and Algamac-fed fish. High prevalence of pink-coloured cyanobacteria (probably *Entophysalis*) and filamentous red algae (*Bonnemaisonia hamifera*) as well as high proportion of "gaped" larvae contributed to the low survival rates of fish. The proportion of fish having a complete pigmentation ranged from 43.1 to 53.1%, and was highest in the menhaden oil-fed fish. Ambicoloration (pigmented on both sides) was also present at considerably high proportions (up to 15.7%). Fish having a complete eye migration accounted for 55.6 to 72.4%, being the highest in Algamac and the lowest in seal oil feeding. A significant difference was, however, observed only in the first experiment. Regression analysis showed that no significant correlation existed between the n-3 HUFA, EFA contents and EFA ratios in rotifers and the normal pigmentation of fish, except for AA in the second experiment, which was found to have a significant positive correlation with the normal

pigmentation of flounder. While the n-3 HUFA, DHA, AA contents and DHA/EPA and DHA/AA ratios correlated positively with the eye migration, the EPA content and EPA/AA ratio correlated negatively with the eye migration of flounder. These relationships were, however, observed only in the first experiment. None of the above diet aspects was found to have relationship with the complete pigmentation + complete eye migration of flounder in this study.

The total lipid content of normally and abnormally pigmented juvenile flounder fed the same diet was similar, but was different among feeding regimes, being higher in seal oil feeding. The pattern in the total lipid content of flounder followed that of the respective *Artemia* diet. The fatty acid composition of total lipid of normally and abnormally pigmented fish was generally similar for each feeding regime. In all feedings, 16:0 and 18:0 were the main saturated fatty acids (SFAs) present. Fish fed seal oil and seal oil+DHASCO had the highest 18:1n-9 content and the lowest amount was observed in Algamac-fed fish. The content of 18:3n-3 was maintained in a relatively narrow range in fish. Flounder fed Algamac-enriched diets contained higher DHA and AA, but lower EPA than fish fed other diets.

The rotifer enrichment study showed that the total lipid content of this prey varied among the enrichment concentration, period and source. The effect of enrichment concentration and period was observed in seal oil, menhaden oil and Algamac feeding treatments, whereas the effect of enrichment period was observed in DHASCO-fed rotifers. The effect of interaction between the enrichment concentration and feeding period was observed only in the seal oil- and menhaden oil-fed rotifers. Fatty acid 16:0

was the dominant SFA, whereas 18:1n-9 and 16:1n-7 were the main component of MUFAs. The PUFAs of rotifers were dominated by DHA and EPA. The proportion of DHA was similar between seal oil- and menhaden oil- and between Algamac- and DHASCO-fed rotifers. Fatty acids 18:3n-3 and 22:5n-3 were also present at concentrations much lower than that of DHA and EPA. Fatty acid 18:2n-6 existed as the primary component of the n-6 family, and generally varied among enrichment concentrations and periods. Arachidonic acid (AA) was present at very low levels (0.37–2.19%) under all concentrations, periods and enrichment sources.

The content of DHA, EPA and AA was affected by both the enrichment concentration and period, except for EPA content of the DHASCO-fed rotifers, which was affected only by the enrichment period. Similarly, the content of DHA, EPA and AA was affected by the interaction between the enrichment concentration and period, except for the DHA content of the Algamac-enriched rotifers. The DHA/EPA ratio in seal oil-fed rotifers generally increased with increasing oil concentration, but was generally unaffected by the enrichment period. In contrast, the EPA/AA and DHA/AA ratios decreased with increasing oil concentration; the pattern of the enrichment period effect was not clear. While the DHA/EPA ratio was unaffected, both EPA/AA and DHA/AA ratios were influenced by the concentration and period in menhaden oil-fed rotifers. The enrichment concentration and period had no effect on the EFA ratios of Algamac- and DHASCO-fed rotifers.

The *Artemia* enrichment study showed that the total lipid contents of prey enriched with various oil emulsions were different among treatments. Both enrichment

concentration and period affected the total lipid content of *Artemia*, although not always in a significant manner. A significant effect of the interaction between the enrichment concentration and period on the lipid content of *Artemia* was also observed.

The SFAs of *Artemia* were composed primarily of 16:0 and 18:0 and their contents were similar among different enrichment concentrations, but varied among enrichment sources. The principal components of MUFAs were 18:1n-9, 16:1n-7 and 18:1n-7. However, variation in their content among different enrichment concentrations was found only for 16:1n-7. The PUFAs of enriched *Artemia* were composed mainly of the n-3 fatty acid series and dominated by 18:3n-3. The content of EPA was highest in menhaden oil- and lowest in DHASCO-fed *Artemia*, whereas that of DHA was highest in DHASCO- and lowest in seal oil-fed *Artemia*. The content of DHA, EPA and AA was affected by both the enrichment concentration and period, except for the AA content of *Artemia* fed menhaden oil which was affected only by the enrichment period. The interaction between the enrichment concentration and feeding period exerted a significant effect on the DHA, EPA and AA content, except on the EPA content of the seal oil-fed *Artemia*. The DHA/EPA ratio remained low (<0.6) in *Artemia* fed with seal oil and menhaden oil emulsions. However, the DHA/EPA, EPA/AA and DHA/AA ratios generally increased, but not always significantly, with increasing enrichment concentration; no clear pattern was observed with the enrichment period.

7.2 Recommendations

1. The enrichment period be reduced to 6 – 12 h for rotifers and to 12 h for *Artemia* from common practice of 24 h. This would allow an increase in DHA content of enriched live feeds comparable to the level achieved under a long (24 h) enrichment period.
2. The concentration of Algamac enrichment medium to enrich rotifers and *Artemia* be reduced from 0.3 to 0.2 g and from 2 to 1 g/million prey, respectively. The manufacturer recommends concentrations of 0.3 g/million rotifers and 2 g/million *Artemia*.
3. For DHASCO, the recommended level is 0.1 g/million rotifers and 0.5 g/million *Artemia*.
4. Seal oil and menhaden oil (~0.2 g/million prey) can be used as cheap sources of marine oils to enrich rotifers, but are not suitable for *Artemia* due to a low DHA/EPA ratio in the final enriched *Artemia*.
5. Further studies are required to determine whether vitamin and mineral supplementation of seal and menhaden oils would improve the performance of yellowtail flounder.
6. Further investigations are needed to determine the optimal levels of dietary n-3 HUFA and essential fatty acids for yellowtail flounder and whether yellowtail flounder is able to metabolically modify dietary essential fatty acids.
7. To determine separately the effects of nutritional composition of enriched rotifers and *Artemia* on flounder performance, such as feeding larvae with different rotifers enrichment diets followed by a standard *Artemia* diet and with a standard rotifers diet followed by different *Artemia* enrichment diets.

8. To determine what exactly constitutes the pink-colored microbial community constantly appearing in the tanks during egg incubation and/or larval rearing and to devise the best possible remedies for controlling this microbial population.

APPENDICES

Appendix 2.1. Fatty acid composition (%) of oils used to prepare emulsions to enrich live feeds during yellowtail flounder (*Limanda ferruginea*) experiments.

Fatty acids	Seal oil		Menhaden oil		Algamac-3010		DHASCO	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
12:0	-	-	-	-	0.54	0.02	5.50	0.06
14:0	4.29	0.13	7.29	0.21	16.5	0.44	15.5	0.12
14:1n-5	0.95	0.01	0.38	0.01	0.11	0.00	0.13	0.00
15:0	0.26	0.01	0.57	0.00	1.00	0.04	-	-
16:0	6.84	0.10	19.07	0.57	40.0	0.87	11.2	0.08
16:1n-7	18.5	0.43	9.69	0.17	2.12	0.07	1.54	0.03
16:2n-4	0.29	0.01	0.33	0.02	-	-	-	-
16:3n-4	0.17	0.01	0.52	0.02	-	-	-	-
16:4n-3	0.16	0.00	0.45	0.08	-	-	-	-
17:0	0.80	0.01	0.36	0.04	0.16	0.02	-	-
17:1	1.26	0.17	1.83	0.28	1.41	0.08	0.18	0.00
18:0	1.22	0.13	3.38	0.10	1.29	0.10	0.90	0.01
18:1n-9	20.8	0.13	10.1	0.38	0.70	0.05	20.5	0.27
18:1n-7	4.70	0.03	3.51	0.33	0.22	0.03	-	-
18:2n-6	1.18	0.67	1.54	0.35	0.34	0.04	0.93	0.03
18:3n-6	1.00	0.85	0.45	0.06	0.28	0.02	-	-
18:3n-3	0.56	0.00	1.61	0.29	0.18	0.03	-	-
18:4n-3	1.35	0.03	2.81	0.21	0.41	0.02	-	-
20:0	0.61	0.04	0.67	0.08	0.48	0.06	-	-
20:1n-11	0.14	0.01	0.35	0.01	-	-	-	-
20:1n-9	10.2	0.31	1.80	0.16	0.45	0.03	-	-
20:2n-6	0.15	0.01	-	-	0.14	0.01	-	-
20:4n-6	0.48	0.03	0.72	0.13	0.47	0.05	-	-
20:4n-3	0.46	0.04	1.70	0.24	0.35	0.02	-	-
20:5n-3	7.67	0.08	11.35	0.48	0.93	0.04	0.13	0.00
22:0	1.48	0.19	0.68	0.01	-	-	0.26	0.00
22:1n-11	0.40	0.03	0.43	0.02	0.39	0.04	-	-
22:2n-6	0.42	0.02	-	-	0.16	0.00	-	-
22:5n-3	4.04	0.11	2.43	0.12	0.36	0.04	0.34	0.01
22:6n-3	8.35	0.26	12.0	0.25	26.8	0.56	41.9	0.43
DHA/EPA	1.09	0.02	1.06	0.02	28.9	0.37	321	14.7
EPA/AA	16.0	0.34	15.8	0.21	1.98	0.13	-	-
DHA/AA	17.4	0.55	16.7	0.63	57.0	1.27	-	-

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. SD, standard deviation; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid; DHASCO, DHA-rich single cell oil.

Appendix 2.2. Fatty acid composition (%) of unenriched rotifers (*Brachionus plicatilis*) used during enrichment experiment with various oil emulsions.

Fatty acid	Mean	SD
14:0	2.08	0.05
14:1n-5	1.02	0.06
15:0	0.41	0.02
16:0	7.43	0.19
16:1n-7	18.58	0.57
16:2n-4	0.73	0.08
16:3n-4	0.46	0.06
17:0	0.28	0.02
17:1	0.73	0.11
18:0	3.34	0.29
18:1n-11	2.28	0.14
18:1n-9	25.32	1.24
18:1n-7	4.44	0.32
18:2n-6	3.70	0.28
18:3n-6	3.02	0.30
18:3n-3	15.32	0.69
18:4n-6	1.22	0.13
18:4n-3	1.46	0.24
20:1n-9	3.63	0.17
20:4n-6	–	–
20:5n-3	2.76	0.37
22:6n-3	–	–

Analyses were carried out in 6 replicates. SD, standard deviation. – not detected.

Appendix 2.3. Fatty acid composition (%) of unenriched *Artemia franciscana* nauplii (6 h post-hatch) used during enrichment experiment with various oil emulsions.

Fatty acid	Mean	SD
14:0	0.71	0.01
14:1n-5	0.84	0.01
15:0	0.58	0.00
16:0	10.32	0.25
16:1n-7	3.17	0.11
16:2n-4	0.57	0.02
16:3n-4	0.84	0.08
16:4n-3	0.61	0.00
17:0	0.71	0.01
17:1	3.48	0.37
18:0	4.99	0.18
18:1n-9	19.21	1.37
18:1n-7	4.88	0.12
18:2n-6	5.03	0.43
18:3n-6	0.32	0.01
18:3n-3	30.03	0.83
18:4n-6	1.00	0.03
18:4n-3	4.67	0.22
20:1n-9	0.81	0.07
20:2n-6	0.73	0.03
20:4n-6	0.71	0.04
20:5n-3	2.82	0.07
22:6n-3	–	–
EPA/AA	3.97	0.34

Analyses were carried out in 6 replicates. SD, standard deviation. – not detected.

Appendix 3.1. Fatty acid composition of total lipids of rotifers (*Brachionus plicatilis*) enriched with different oil emulsions used in the first experiment with yellowtail flounder (*Limanda ferruginea*).

Fatty acid	Rotifers enriched with:					
	Seal oil		Menhaden oil		Algamac-3010	
	Mean	SD	Mean	SD	Mean	SD
12:0	0.11^{a}	0.01	0.12^{a}	0.01	0.23^{b}	0.02
14:0	2.73^{a}	0.16	3.84^{b}	0.12	4.48^{c}	0.30
14:1n-5	0.75^{a}	0.07	1.01^{b}	0.01	0.80^{a}	0.03
15:0	0.36^{a}	0.05	0.53^{b}	0.04	0.63^{b}	0.08
16:0	6.76^{a}	0.39	11.96^{b}	0.54	12.14^{b}	0.48
16:1n-7	16.32^{a}	0.61	13.73^{b}	0.26	10.59^{c}	0.64
16:2n-4	0.37^{a}	0.04	0.58^{b}	0.03	0.45^{a}	0.04
16:3n-4	0.24^{a}	0.05	0.40^{b}	0.04	0.29^{a}	0.01
16:4n-3	0.46^{a}	0.02	1.15^{b}	0.08	0.18^{c}	0.00
17:0	0.20^{a}	0.02	0.36^{b}	0.05	0.46^{c}	0.01
17:1	0.46^{a}	0.02	0.90^{b}	0.10	0.41^{a}	0.02
18:0	2.16^{a}	0.07	3.37^{b}	0.11	2.76^{c}	0.15
18:1n-11	-	-	1.80^{a}	0.06	2.10^{b}	0.03
18:1n-9	24.41^{a}	0.68	17.11^{b}	0.31	12.71^{c}	0.59
18:1n-7	4.47^{a}	0.31	4.55^{a}	0.28	3.68^{b}	0.22
18:2n-6	3.02^{ab}	0.23	2.66^{a}	0.13	3.20^{b}	0.22
18:3n-6	0.13^{a}	0.00	0.17^{b}	0.01	0.21^{c}	0.01
18:3n-3	0.60^{a}	0.06	0.86^{b}	0.09	0.17^{c}	0.01
18:4n-6	0.77^{a}	0.04	1.28^{b}	0.04	0.47^{c}	0.04
18:4n-3	0.16^{a}	0.02	0.14^{a}	0.01	0.22^{b}	0.02
20:0	0.60^{a}	0.04	1.01^{b}	0.07	1.01^{b}	0.08
20:1n-11	0.67^{a}	0.02	0.60^{b}	0.01	0.64^{ab}	0.02
20:1n-9	6.19^{a}	0.13	2.95^{b}	0.08	2.01^{c}	0.02
20:2n-6	0.46^{a}	0.03	0.39^{a}	0.01	0.86^{b}	0.05
20:4n-6	1.35^{a}	0.19	1.68^{b}	0.09	2.29^{c}	0.01
20:4n-3	0.69^{a}	0.02	1.56^{b}	0.11	0.80^{a}	0.05
20:5n-3	5.90^{a}	0.11	6.49^{b}	0.13	3.70^{c}	0.18
22:0	0.42^{a}	0.04	0.16^{b}	0.01	0.18^{b}	0.02
22:1n-11	0.69^{a}	0.04	0.17^{b}	0.01	0.21^{b}	0.02
22:1n-9	1.00^{a}	0.02	0.99^{a}	0.04	1.57^{b}	0.10
22:2n-6	0.24^{a}	0.01	0.35^{b}	0.04	0.35^{b}	0.03
22:4n-6	0.20^{a}	0.02	0.48^{b}	0.02	0.42^{c}	0.01
22:4n-3	0.54^{a}	0.01	0.49^{b}	0.03	0.18^{c}	0.00
22:5n-6	0.10^{a}	0.01	0.16^{a}	0.01	1.31^{b}	0.07
22:5n-3	3.96^{a}	0.15	2.25^{b}	0.08	0.33^{c}	0.04
22:6n-3	7.09^{a}	0.33	6.62^{a}	0.27	22.31^{b}	0.73
DHA/EPA	1.20^{a}	0.03	1.02^{b}	0.06	6.04^{c}	0.09
EPA/AA	4.42^{a}	0.71	3.87^{a}	0.27	1.61^{b}	0.08
DHA/AA	5.32^{a}	0.99	3.94^{a}	0.04	9.72^{b}	0.35

Values are means and standard deviation (SD) of triplicate determinations. – Mean values <0.1 or not detected. SD values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another.

Appendix 3.2. Fatty acid composition of total lipids of *Artemia franciscana* enriched with different oil emulsions used in the first experiment with yellowtail flounder (*Limanda ferruginea*).

Fatty acids	*Artemia* enriched with:					
	Seal oil		Menhaden oil		Algamac-3010	
	Mean	SD	Mean	SD	Mean	SD
14:0	1.49^{a}	0.06	1.69^{a}	0.15	2.19^{b}	0.28
14:1n-5	0.95^{a}	0.03	1.04^{a}	0.04	0.74^{b}	0.04
15:0	0.42^{a}	0.12	0.45^{a}	0.08	0.41^{a}	0.12
16:0	9.92^{a}	0.28	12.33^{b}	0.30	11.43^{b}	0.85
16:1n-7	8.17^{a}	0.22	5.84^{b}	0.53	4.02^{c}	0.31
16:2n-4	0.47^{a}	0.03	0.58^{b}	0.04	0.43^{a}	0.02
16:3n-4	0.85^{a}	0.17	0.93^{a}	0.18	0.89^{a}	0.18
17:0	0.60^{ab}	0.11	0.70^{a}	0.03	0.47^{b}	0.03
17:1	0.87^{a}	0.03	1.05^{b}	0.02	1.01^{b}	0.05
18:0	3.93^{a}	0.13	4.92^{b}	0.21	3.74^{a}	0.27
18:1n-11	0.54^{a}	0.09	0.54^{a}	0.02	0.69^{b}	0.04
18:1n-9	21.88^{a}	0.25	19.77^{b}	0.39	13.42^{c}	0.55
18:1n-7	6.56^{a}	0.19	6.91^{a}	0.10	5.13^{b}	0.29
18:2n-6	5.82^{a}	0.58	5.67^{a}	0.14	4.17^{b}	0.33
18:3n-3	19.96^{a}	0.43	24.68^{b}	0.54	18.96^{a}	0.99
18:4n-6	2.87^{a}	0.07	3.53^{b}	0.08	2.49^{a}	0.29
20:1n-9	2.53^{a}	0.07	-	-	-	-
20:4n-6	1.02^{a}	0.02	1.10^{a}	0.01	2.93^{b}	0.06
20:5n-3	7.79^{a}	0.48	6.53^{ab}	0.76	6.43^{b}	0.28
22:5n-3	1.26^{a}	0.02	-	-	5.39^{c}	0.50
22:6n-3	2.65^{a}	0.13	3.50^{b}	0.03	15.22^{c}	1.20
DHA/EPA	0.35^{a}	0.05	0.48^{a}	0.04	2.37^{b}	0.07
EPA/AA	7.62^{a}	0.23	5.95^{b}	0.19	2.19^{c}	0.10
DHA/AA	2.59^{a}	0.28	3.19^{b}	0.23	5.19^{c}	0.13

Values are means and standard deviation (SD) of 5 replicates. – Mean values <0.1 or not detected. Values in each row with different superscripts are different ($p<0.05$) from one another.

Appendix 3.3. Fatty acid composition of total lipids of rotifers (*Brachionus plicatilis*) enriched with different oil emulsions used in the second experiment with yellowtail flounder (*Limanda ferruginea*).

Fatty acid	Rotifers enriched with:					
	Seal oil		Seal oil+DHASCO		Algamac-3010	
	Mean	SD	Mean	SD	Mean	SD
12:0	0.10[a]	0.01	0.21[b]	0.01	0.16[c]	0.01
14:0	2.59[a]	0.14	3.14[b]	0.15	5.09[c]	0.26
14:1n-5	0.78[a]	0.02	0.74[a]	0.03	0.58[b]	0.01
15:0	0.31[a]	0.02	0.30[a]	0.01	0.43[b]	0.01
16:0	6.10[a]	0.28	6.85[a]	0.39	18.07[b]	0.48
16:1n-7	14.68[a]	0.20	11.89[b]	0.16	7.25[c]	0.26
16:2n-4	0.40[a]	0.03	0.45[a]	0.02	0.26[b]	0.03
16:3n-4	0.40[a]	0.04	0.17[b]	0.00	0.22[b]	0.01
16:4n-3	0.16[a]	0.00	0.25[b]	0.00	0.12[c]	0.01
17:0	0.43[a]	0.01	0.34[b]	0.01	0.34[b]	0.01
17:1	0.72[a]	0.03	1.49[b]	0.08	1.46[b]	0.11
18:0	1.67[a]	0.10	2.07[b]	0.13	2.28[b]	0.11
18:1n-11	-	-	-	-	1.34[b]	0.06
18:1n-9	23.64[a]	0.32	24.01[a]	0.59	9.47[b]	0.31
18:1n-7	4.09[a]	0.27	3.55[b]	0.17	2.70[c]	0.11
18:2n-6	5.96[a]	0.24	6.12[a]	0.43	3.92[b]	0.16
18:3n-6	0.40[a]	0.03	0.42[a]	0.03	0.45[a]	0.01
18:3n-3	0.98[a]	0.02	0.87[b]	0.03	0.55[c]	0.01
18:4n-6	0.74[a]	0.02	0.44[b]	0.01	0.21[c]	0.01
18:4n-3	0.10[a]	0.00	0.13[b]	0.00	0.21[c]	0.01
20:0	0.56[a]	0.02	0.72[b]	0.04	0.86[c]	0.07
20:1n-11	0.51[a]	0.02	0.60[b]	0.03	0.39[c]	0.01
20:1n-9	7.42[a]	0.27	5.98[b]	0.32	1.95[c]	0.15
20:2n-6	0.26[a]	0.02	0.36[b]	0.01	0.52[c]	0.01
20:4n-6	0.76[a]	0.01	0.54[b]	0.01	1.65[c]	0.09
20:4n-3	0.69[a]	0.01	0.54[b]	0.01	0.87[c]	0.02
20:5n-3	5.83[a]	0.16	3.89[b]	0.18	3.10[c]	0.14
22:0	0.11[a]	0.00	0.10[a]	0.00	0.13[a]	0.00
22:1n-11	0.95[a]	0.02	0.72[b]	0.01	0.28[c]	0.01
22:1n-9	0.91[a]	0.03	0.94[a]	0.01	0.59[b]	0.04
22:2n-6	0.19[a]	0.01	0.23[a]	0.00	0.33[b]	0.03
22:4n-6	0.17[a]	0.01	0.11[b]	0.01	0.31[c]	0.01
22:4n-3	0.25[a]	0.01	0.18[b]	0.01	0.45[c]	0.02
22:5n-6	0.13[a]	0.01	0.23[b]	0.01	0.18[c]	0.01
22:5n-3	3.99[a]	0.27	2.77[b]	0.22	1.02[c]	0.06
22:6n-3	6.86[a]	0.25	11.18[b]	0.56	24.83[c]	0.74
24:1	0.13[a]	0.01	-	-	0.10[b]	0.00
DHA/EPA	1.18[a]	0.07	2.88[b]	0.08	8.02[c]	0.11
EPA/AA	7.66[a]	0.12	7.23[b]	0.12	1.87[c]	0.06
DHA/AA	9.02[a]	0.34	20.78[b]	0.43	15.01[c]	0.34

Values are means and standard deviation (SD) of triplicate determinations. – Mean values <0.1 or not detected. SD values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHASCO, DHA-rich single cell oil.

Appendix 3.4. Fatty acid composition of total lipids of *Artemia franciscana* enriched with different oil emulsions used in the second experiment with yellowtail flounder (*Limanda ferruginea*).

Fatty acid	Enrichment media					
	Seal oil		Seal oil + DHASCO		Algamac-3010	
	Mean	SD	Mean	SD	Mean	SD
12:0	-	-	-	-	0.19^{b}	0.02
14:0	1.30^{a}	0.02	2.07^{b}	0.03	1.81^{c}	0.02
14:1n-5	0.59^{a}	0.01	0.39^{b}	0.00	0.57^{a}	0.01
15:0	0.36^{a}	0.00	0.34^{b}	0.00	0.35^{ab}	0.01
16:0	10.98^{a}	0.00	12.86^{b}	0.15	11.39^{c}	0.09
16:1n-7	6.82^{a}	0.02	5.27^{b}	0.09	5.88^{c}	0.05
16:2n-4	0.49^{a}	0.01	0.34^{b}	0.01	0.53^{a}	0.09
16:3n-4	0.73^{a}	0.01	0.50^{b}	0.06	0.61^{c}	0.04
16:4n-3	0.17^{a}	0.00	0.50^{b}	0.02	0.14^{a}	0.01
17:0	0.77^{a}	0.00	0.72^{a}	0.08	0.76^{a}	0.02
17:1	1.33^{a}	0.02	0.78^{b}	0.00	0.96^{c}	0.02
18:0	4.07^{a}	0.03	3.40^{b}	0.06	3.93^{c}	0.04
18:1n-9	23.01^{a}	0.14	23.46^{a}	1.02	18.19^{b}	0.22
18:1n-7	5.93^{a}	0.02	4.37^{b}	0.07	5.57^{c}	0.03
18:2n-6	5.34^{a}	0.03	5.94^{b}	0.02	5.19^{c}	0.07
18:3n-6	0.84^{a}	0.00	0.95^{b}	0.01	0.88^{c}	0.01
18:3n-3	13.94^{a}	0.03	13.07^{b}	0.11	14.39^{c}	0.11
18:4n-6	2.57^{a}	0.01	2.50^{b}	0.00	2.66^{c}	0.02
18:4n-3	0.14^{a}	0.00	0.11^{b}	0.00	0.14^{c}	0.00
20:0	0.22^{a}	0.00	-	-	0.18^{b}	0.00
20:1n-11	0.14^{a}	0.01	-	-	0.13^{a}	0.00
20:1n-9	3.22^{a}	0.01	2.71^{b}	0.08	2.34^{c}	0.02
20:2n-6	0.17^{a}	0.00	0.33^{b}	0.01	0.16^{a}	0.04
20:4n-6	0.69^{a}	0.01	0.61^{b}	0.02	0.79^{c}	0.01
20:4n-3	0.40^{a}	0.00	0.76^{b}	0.00	0.40^{a}	0.00
20:5n-3	4.17^{a}	0.04	4.85^{b}	0.09	4.13^{a}	0.04
22:0	0.44^{a}	0.00	0.40^{b}	0.00	0.45^{a}	0.01
22:1n-11	-	-	-	-	0.15^{a}	0.01
22:1n-9	0.42^{a}	0.01	0.36^{b}	0.02	0.29^{c}	0.00
22:2n-6	0.19^{a}	0.03	0.14^{a}	0.00	0.21^{a}	0.02
22:4n-6	0.70^{a}	0.02	0.16^{b}	0.00	0.38^{c}	0.01
22:4n-3	0.85^{a}	0.02	0.16^{b}	0.03	0.37^{c}	0.01
22:5n-3	1.07^{a}	0.04	0.26^{b}	0.01	0.77^{c}	0.08
22:6n-3	2.22^{a}	0.02	5.31^{b}	0.02	12.01^{c}	0.25
24:1	0.11^{a}	0.01	-	-	0.10^{a}	0.00
DHA/EPA	0.53^{a}	0.00	1.09^{b}	0.02	2.91^{c}	0.04
EPA/AA	6.06^{a}	0.03	7.95^{b}	0.05	5.20^{c}	0.00
DHA/AA	3.22^{a}	0.02	8.71^{b}	0.03	15.20^{c}	0.23

Values are means and standard deviation (SD) of triplicate determinations. – Mean values <0.1 or not detected. S.D. values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHASCO, DHA-rich single cell oil.

Appendix 3.5. Fatty acid composition of total lipids of uneaten *Artemia franciscana* used in yellowtail flounder (*Limanda ferruginea*) feeding experiments.

Fatty acids	*Artemia* enriched with:					
	Seal oil		Menhaden oil		Algamac-3010	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
14:0	1.50	0.10	1.51	0.25	1.55	0.08
14:1n-5	0.74	0.04	0.74	0.06	0.65	0.10
15:0	0.10^{a}	0.02	0.38^{b}	0.03	0.26^{c}	0.03
16:0	10.22^{a}	0.59	12.98^{b}	0.89	10.74^{a}	0.25
16:1n-7	8.56^{a}	0.50	4.60^{b}	0.44	3.70^{c}	0.29
16:2n-5	0.44^{a}	0.05	0.27^{b}	0.02	0.44^{a}	0.04
16:3n-3	0.67	0.05	0.71	0.05	0.64	0.05
17:0	0.32	0.04	0.35	0.05	0.31	0.02
17:1	0.89^{a}	0.05	0.62^{b}	0.07	0.58^{b}	0.04
18:0	5.03^{a}	0.39	8.11^{b}	1.15	5.00^{a}	0.28
18:1n-11	-	-	-	-	0.35^{a}	0.04
18:1n-9	23.96^{a}	0.26	20.41^{b}	0.50	15.09^{c}	0.70
18:1n-7	7.34^{a}	0.51	7.75^{b}	0.70	6.00^{c}	0.62
18:2n-6	5.27^{a}	0.41	5.29^{a}	0.47	4.35^{b}	0.35
18:3n-3	21.50^{a}	0.94	24.17^{b}	0.59	19.25^{a}	0.90
18:4n-3	1.93^{a}	0.40	3.20^{b}	0.21	2.29^{a}	0.20
20:0	1.84^{a}	0.31	-	-	-	-
20:4n-6	0.43^{a}	0.03	0.46^{a}	0.05	3.95^{b}	0.16
20:5n-3	8.16^{a}	0.49	6.72^{b}	0.37	8.53^{a}	0.39
22:5n-3	-	-	-	-	4.61^{a}	0.41
22:6n-3	0.32^{a}	0.06	0.86^{b}	0.12	11.54^{c}	1.31
DHA/EPA	0.04^{a}	0.01	0.13^{b}	0.02	1.36^{c}	0.17
EPA/AA	19.09^{a}	1.79	14.78^{b}	1.80	2.16^{c}	0.13
DHA/AA	0.75^{a}	0.15	1.89^{b}	0.34	2.93^{c}	0.35

Values are means and standard deviation (SD) of 5 replicates. – Mean values <0.1 or not detected.
Values in each row with different superscripts are different ($p<0.05$) from one another.

Appendix 5.1. Fatty acid composition (%) of total lipids of rotifers (*Brachionus plicatilis*) enriched for 6 hours with seal oil emulsion at different concentrations.

Fatty acid	Emulsion concentrations (g/million rotifers)							
	0.2		0.4		0.6		0.8	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14:0	2.75^{a}	0.14	2.98^{ab}	0.11	3.02^{ab}	0.15	3.13^{b}	0.11
14:1n-5	0.77	0.02	0.76	0.04	0.78	0.06	0.85	0.06
15:0	0.34	0.04	0.32	0.01	0.35	0.03	0.36	0.02
16:0	7.81	0.28	7.40	0.31	7.14	0.21	7.06	0.36
16:1n-7	20.02	0.53	21.04	0.46	20.59	0.36	20.58	0.60
16:2n-4	0.58^{ab}	0.04	0.63^{a}	0.05	0.52^{b}	0.05	0.64^{a}	0.02
16:3n-4	0.27^{a}	0.01	0.26^{a}	0.01	0.21^{b}	0.01	0.34^{c}	0.01
16:4n-3	0.39^{a}	0.02	0.40^{a}	0.04	0.42^{a}	0.01	0.54^{b}	0.01
17:0	0.80	0.05	0.89	0.06	0.85	0.04	0.93	0.06
17:1	1.07	0.07	1.05	0.11	1.19	0.09	1.15	0.07
18:0	2.45^{a}	0.12	2.28^{a}	0.11	3.83^{b}	0.13	2.94^{c}	0.14
18:1n-9	25.85	0.51	25.28	0.41	24.99	0.27	24.68	0.61
18:1n-7	3.10	0.09	3.35	0.22	3.11	0.17	3.41	0.16
18:2n-6	4.11	0.14	4.43	0.22	4.54	0.15	4.34	0.21
18:3n-6	1.91	0.05	1.91	0.11	1.79	0.10	1.68	0.11
18:3n-3	3.87^{a}	0.10	3.50^{b}	0.10	3.52^{b}	0.09	3.29^{b}	0.15
18:4n-3	0.51^{a}	0.02	0.62^{b}	0.05	0.42^{c}	0.01	0.75^{d}	0.02
20:0	0.50	0.02	0.58	0.03	0.62	0.05	0.61	0.07
20:1n-11	0.29^{a}	0.02	0.40^{b}	0.03	0.54^{c}	0.03	0.46^{bc}	0.05
20:1n-9	4.71	0.13	4.89	0.12	4.94	0.23	4.60	0.12
20:2n-6	0.45	0.04	0.51	0.02	0.48	0.04	0.48	0.03
20:4n-6	0.69	0.03	0.71	0.04	0.71	0.05	0.79	0.05
20:4n-3	0.43	0.01	0.46	0.02	0.44	0.02	0.48	0.05
20:5n-3	4.75^{a}	0.16	4.78^{a}	0.20	4.12^{b}	0.23	4.01^{b}	0.24
22:0	0.13	0.00	0.14	0.01	0.13	0.01	0.15	0.01
22:1n-11	0.50^{ab}	0.03	0.48^{ab}	0.04	0.42^{a}	0.03	0.52^{b}	0.04
22:2n-6	0.48^{ac}	0.03	0.35^{b}	0.03	0.52^{a}	0.01	0.45^{c}	0.03
22:4n-6	0.14^{ab}	0.00	0.12^{a}	0.01	0.15^{b}	0.00	0.13^{ab}	0.01
22:4n-3	0.18	0.01	0.16	0.02	0.19	0.01	0.21	0.03
22:5n-3	2.95^{a}	0.10	2.22^{b}	0.12	2.20^{b}	0.11	2.90^{a}	0.13
22:6n-3	3.94^{a}	0.15	4.71^{b}	0.22	4.50^{ab}	0.30	4.86^{b}	0.26
DHA/EPA	0.83^{a}	0.08	0.98^{ab}	0.11	1.10^{ab}	0.14	1.21^{b}	0.09
EPA/AA	6.92^{a}	0.14	6.72^{a}	0.07	5.80^{b}	0.12	5.10^{c}	0.22
DHA/AA	5.74^{a}	0.16	6.61^{b}	0.33	6.35^{ab}	0.41	6.18^{ab}	0.31

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. Standard deviation (SD) values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Appendix 5.2. Fatty acid composition (%) of total lipids of rotifers (*Brachionus plicatilis*) enriched for 12 hours with seal oil emulsion at different concentrations.

Fatty acid	Emulsion concentrations (g/million rotifers)							
	0.2		0.4		0.6		0.8	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14:0	3.01^{ab}	0.12	2.96^{a}	0.07	3.08^{ab}	0.19	3.33^{b}	0.11
14:1n-5	0.64^{a}	0.08	0.76^{ab}	0.06	0.85^{b}	0.07	0.82^{b}	0.01
15:0	0.36	0.04	0.33	0.02	0.33	0.02	0.36	0.02
16:0	6.93	0.10	6.97	0.22	6.61	0.38	6.80	0.11
16:1n-7	18.68	0.32	18.25	0.40	18.19	0.85	19.38	0.28
16:2n-4	0.28^{a}	0.02	0.36^{b}	0.03	0.34^{bc}	0.01	0.31^{ac}	0.00
16:3n-4	0.17^{a}	0.01	0.17^{a}	0.01	0.21^{b}	0.00	0.21^{b}	0.01
16:4n-3	0.39^{a}	0.02	0.32^{b}	0.04	0.42^{a}	0.01	0.39^{a}	0.01
17:0	0.54^{a}	0.05	0.79^{b}	0.06	0.84^{b}	0.06	0.82^{b}	0.08
17:1	0.46^{a}	0.06	0.53^{ab}	0.05	0.54^{b}	0.08	0.56^{b}	0.07
18:0	2.57^{a}	0.05	2.85^{b}	0.07	2.47^{a}	0.11	2.39^{a}	0.10
18:1n-9	24.72	0.42	24.94	0.67	24.28	1.05	24.45	0.41
18:1n-7	3.44	0.21	3.52	0.16	3.54	0.19	3.56	0.27
18:2n-6	4.36	0.17	4.45	0.10	4.38	0.20	4.60	0.13
18:3n-6	1.72^{a}	0.06	1.62^{a}	0.02	1.39^{b}	0.06	1.41^{b}	0.03
18:3n-3	3.47^{a}	0.06	3.23^{ab}	0.10	3.05^{b}	0.18	3.19^{ab}	0.04
18:4n-3	0.25^{a}	0.03	0.53^{b}	0.04	0.83^{c}	0.06	0.69^{d}	0.05
20:0	0.68^{a}	0.06	0.97^{b}	0.07	0.80^{ab}	0.08	0.71^{a}	0.07
20:1n-11	0.56	0.06	0.51	0.06	0.51	0.06	0.52	0.01
20:1n-9	4.96	0.15	5.18	0.16	5.04	0.33	5.22	0.15
20:2n-6	0.20	0.01	0.19	0.01	0.19	0.00	0.20	0.01
20:4n-6	0.73	0.04	0.87	0.08	0.87	0.09	0.87	0.04
20:4n-3	0.58^{a}	0.06	0.47^{b}	0.03	0.61^{a}	0.04	0.61^{a}	0.01
20:5n-3	5.38	0.12	5.36	0.17	4.91	0.26	5.08	0.16
22:0	0.13^{a}	0.01	0.10^{b}	0.01	0.12^{ab}	0.01	0.13^{a}	0.00
22:1n-13	0.76^{a}	0.07	0.42^{b}	0.03	0.46^{b}	0.01	0.59^{c}	0.01
22:1n-11	0.81	0.06	1.03	0.13	0.90	0.11	0.97	0.10
22:2n-6	0.36	0.04	0.32	0.02	0.33	0.01	0.31	0.01
22:4n-6	0.18^{ab}	0.01	0.16^{ac}	0.01	0.19^{b}	0.01	0.14^{c}	0.00
22:4n-3	0.21^{a}	0.01	0.14^{b}	0.01	0.15^{b}	0.00	0.19^{a}	0.01
22:5n-3	3.45^{ab}	0.09	3.43^{ab}	0.10	3.41^{a}	0.13	3.70^{b}	0.10
22:6n-3	4.92	0.33	5.23	0.29	5.18	0.17	5.39	0.17
DHA/EPA	0.92	0.10	0.98	0.12	1.06	0.11	1.06	0.13
EPA/AA	7.38^{a}	0.46	6.16^{b}	0.25	5.64^{a}	0.52	5.87^{b}	0.16
DHA/AA	6.76	0.78	6.01	0.45	5.95	0.27	6.22	0.34

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. Standard deviation (SD) values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Appendix 5.3. Fatty acid composition (%) of total lipids of rotifers (*Brachionus plicatilis*) enriched for 18 hours with seal oil emulsion at different concentrations.

Fatty acid	Emulsion concentrations (g/million rotifers)							
	0.2		0.4		0.6		0.8	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
12:0	0.14^{a}	0.01	0.10^{b}	0.01	0.11^{b}	0.01	0.12^{ab}	0.01
14:0	3.13^{a}	0.03	3.28^{b}	0.00	3.02^{c}	0.03	3.33^{b}	0.06
14:1n-5	0.80	0.13	0.92	0.00	0.86	0.02	0.89	0.14
15:0	0.32^{a}	0.01	0.30^{ab}	0.01	0.29^{b}	0.01	0.31^{ab}	0.00
16:0	6.27^{a}	0.06	5.69^{b}	0.01	5.52^{b}	0.07	6.19^{a}	0.12
16:1n-7	19.71	0.50	20.67	0.64	20.07	0.77	21.00	0.46
16:2n-4	0.28^{ab}	0.01	0.33^{a}	0.00	0.26^{b}	0.05	0.34^{a}	0.01
16:3n-4	0.32^{a}	0.01	0.30^{ab}	0.01	0.28^{b}	0.02	0.31^{ab}	0.00
16:4n-3	0.23^{ab}	0.01	0.24^{ab}	0.01	0.21^{a}	0.02	0.25^{b}	0.00
17:0	0.70^{a}	0.01	0.79^{b}	0.01	0.71^{a}	0.01	0.80^{b}	0.01
17:1	0.69^{a}	0.01	0.59^{b}	0.01	0.63^{ab}	0.02	0.65^{b}	0.05
18:0	1.99^{a}	0.01	1.80^{b}	0.04	1.81^{b}	0.03	1.95^{a}	0.03
18:1n-9	25.18	0.50	22.85	0.83	24.81	1.97	22.84	0.79
18:1n-7	3.49	0.02	3.73	0.10	3.41	0.04	3.73	0.29
18:2n-6	2.80^{a}	0.02	3.00^{b}	0.01	2.86^{a}	0.02	2.98^{b}	0.06
18:3n-6	0.13	0.00	0.14	0.00	0.13	0.00	0.14	0.00
18:3n-3	0.55^{a}	0.00	0.63^{b}	0.00	0.59^{c}	0.00	0.64^{b}	0.01
20:0	0.71	0.04	0.64	0.01	0.65	0.04	0.66	0.01
20:1n-11	0.43^{a}	0.04	0.45^{ac}	0.00	0.53^{b}	0.01	0.49^{bc}	0.01
20:1n-9	5.97^{a}	0.02	5.42^{b}	0.02	5.19^{c}	0.12	5.58^{b}	0.11
20:2n-6	0.45^{a}	0.04	0.22^{b}	0.00	0.39^{c}	0.01	0.22^{b}	0.00
20:4n-6	1.00^{a}	0.08	1.00^{a}	0.00	1.08^{b}	0.07	0.97^{a}	0.02
20:4n-3	0.71^{a}	0.00	0.85^{b}	0.00	0.86^{b}	0.04	0.90^{b}	0.02
20:5n-3	6.15^{a}	0.17	7.44^{b}	0.10	7.17^{b}	0.12	6.47^{a}	0.14
22:1n-13	0.31^{a}	0.01	0.57^{b}	0.01	0.50^{c}	0.01	0.57^{b}	0.02
22:1n-11	0.74^{a}	0.02	0.65^{b}	0.00	0.67^{b}	0.01	0.66^{b}	0.02
22:2n-6	0.54^{a}	0.00	0.48^{b}	0.01	0.55^{a}	0.01	0.49^{b}	0.00
22:4n-6	0.16	0.00	0.13	0.01	0.15	0.02	0.15	0.01
22:4n-3	0.20	0.01	0.20	0.02	0.20	0.01	0.22	0.01
22:5n-6	-	-	-	-	-	-	0.12^{a}	0.05
22:5n-3	3.87^{a}	0.06	4.26^{b}	0.02	4.29^{b}	0.09	4.84^{c}	0.27
22:6n-3	7.06^{a}	0.19	8.27^{b}	0.12	8.29^{b}	0.22	6.62^{a}	0.19
DHA/EPA	1.15^{a}	0.00	1.11^{b}	0.00	1.16^{a}	0.01	1.02^{c}	0.01
EPA/AA	6.15^{a}	0.57	7.43^{b}	0.03	6.66^{a}	0.34	6.70^{a}	0.00
DHA/AA	7.07^{ac}	0.66	8.25^{b}	0.05	7.69^{a}	0.31	6.85^{c}	0.06

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. Standard deviation (SD) values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Appendix 5.4. Fatty acid composition (%) of total lipids of rotifers (*Brachionus plicatilis*) enriched for 24 hours with seal oil emulsion at different concentrations.

Fatty acid	Emulsion concentrations (g/million rotifers)							
	0.2		0.4		0.6		0.8	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
12:0	0.14	0.01	0.15	0.01	0.14	0.01	0.17	0.02
14:0	2.95^{a}	0.06	2.63^{b}	0.15	2.99^{a}	0.07	3.03^{a}	0.11
14:1n-5	0.68^{a}	0.03	0.94^{b}	0.04	1.04^{c}	0.04	1.02^{bc}	0.04
15:0	0.32	0.02	0.36	0.05	0.33	0.05	0.38	0.01
16:0	7.82	0.50	7.24	0.39	7.54	0.27	8.19	0.35
16:1n-7	16.71^{ab}	0.28	15.61^{a}	0.38	17.16^{b}	0.71	16.52^{ab}	0.49
16:2n-4	0.34^{a}	0.02	0.47^{b}	0.02	0.42^{c}	0.01	0.40^{c}	0.01
16:3n-4	0.19^{a}	0.03	0.23^{ab}	0.01	0.25^{b}	0.01	0.26^{b}	0.01
16:4n-3	0.46^{ac}	0.03	0.43^{a}	0.01	0.53^{bd}	0.01	0.50^{cd}	0.02
17:0	0.18	0.03	0.22	0.01	0.19	0.01	0.21	0.01
17:1	0.47	0.04	0.45	0.03	0.48	0.02	0.49	0.02
18:0	2.27	0.12	2.52	0.06	2.51	0.10	2.52	0.18
18:1n-9	23.61	0.78	25.39	0.67	25.01	1.08	24.29	0.58
18:1n-7	4.15	0.25	4.49	0.24	4.78	0.41	4.69	0.28
18:2n-6	3.41	0.36	3.11	0.32	3.11	0.17	3.26	0.11
18:3n-6	0.14^{a}	0.01	0.11^{b}	0.01	0.12^{b}	0.01	0.12^{b}	0.00
18:3n-3	0.66^{a}	0.03	0.47^{b}	0.04	0.63^{a}	0.04	0.60^{a}	0.02
18:4n-6	0.23^{a}	0.01	0.44^{b}	0.02	0.33^{c}	0.02	0.27^{a}	0.01
20:0	0.79^{a}	0.07	1.27^{b}	0.05	1.03^{c}	0.06	0.89^{ac}	0.03
20:1n-11	0.70	0.04	0.65	0.04	0.66	0.04	0.66	0.02
20:1n-9	6.88	0.41	6.44	0.18	6.38	0.38	6.81	0.30
20:2n-6	0.25	0.03	0.27	0.02	0.24	0.01	0.25	0.01
20:4n-6	0.93^{a}	0.07	1.13^{b}	0.05	1.11^{b}	0.04	1.09^{b}	0.04
20:4n-3	0.69^{ab}	0.03	0.68^{a}	0.05	0.79^{b}	0.05	0.77^{ab}	0.03
20:5n-3	5.95	0.26	5.92	0.39	5.92	0.26	5.80	0.29
22:1n-13	0.90^{a}	0.04	0.59^{b}	0.05	0.60^{b}	0.04	0.74^{c}	0.03
22:1n-11	0.97^{a}	0.04	1.33^{b}	0.08	1.16^{c}	0.07	1.23^{bc}	0.04
22:2n-6	0.47	0.05	0.44	0.03	0.42	0.03	0.39	0.01
22:4n-6	0.23	0.02	0.24	0.05	0.25	0.02	0.18	0.01
22:4n-3	0.26^{a}	0.02	0.21^{ab}	0.03	0.20^{b}	0.01	0.23^{ab}	0.01
22:5n-6	-	-	-	-	0.10^{a}	0.01	0.11^{a}	0.00
22:5n-3	3.96	0.20	4.27	0.27	4.05	0.24	3.69	0.13
22:6n-3	6.85	0.64	6.58	0.56	5.56	0.43	5.50	0.42
DHA/EPA	1.15^{a}	0.06	1.11^{a}	0.02	0.94^{b}	0.03	0.95^{b}	0.02
EPA/AA	6.42^{a}	0.22	5.23^{b}	0.46	5.36^{b}	0.06	5.30^{b}	0.08
DHA/AA	7.38^{a}	0.13	5.82^{b}	0.63	5.03^{b}	0.23	5.03^{b}	0.20

Analyses was carried out in triplicates. – Mean values <0.1 or not detected. Standard deviation (SD) values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Appendix 5.5. Fatty acid composition (%) of total lipids of rotifers (*Brachionus plicatilis*) enriched for 6 hours with menhaden oil emulsion at different concentrations.

Fatty acid	Emulsion concentrations (g/million rotifers)							
	0.2		0.4		0.6		0.8	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
12:0	0.15^{a}	0.00	0.21^{b}	0.00	0.18^{c}	0.02	0.17^{ac}	0.00
14:0	3.79^{a}	0.13	4.26^{b}	0.06	4.19^{b}	0.04	4.27^{b}	0.01
14:1n-5	0.76^{ab}	0.02	0.77^{a}	0.00	0.74^{b}	0.01	0.74^{b}	0.00
15:0	0.52^{a}	0.01	0.57^{b}	0.01	0.52^{a}	0.01	0.50^{a}	0.01
16:0	9.26^{a}	0.29	8.85^{ab}	0.05	8.77^{b}	0.11	8.64^{b}	0.05
16:1n-7	17.13^{a}	0.57	18.13^{b}	0.20	17.76^{ab}	0.22	18.16^{b}	0.02
16:2n-4	0.52	0.02	0.50	0.01	0.49	0.01	0.49	0.00
16:3n-4	0.34^{a}	0.03	0.36^{a}	0.03	0.37^{a}	0.04	0.47^{b}	0.02
16:4n-3	1.30^{a}	0.04	1.55^{b}	0.01	1.58^{b}	0.02	1.71^{c}	0.02
17:0	0.33^{a}	0.01	0.29^{b}	0.00	0.28^{b}	0.01	0.27^{b}	0.01
17:1	1.40^{a}	0.05	1.62^{ab}	0.10	1.56^{ab}	0.09	1.72^{b}	0.11
18:0	2.93^{a}	0.07	2.57^{b}	0.06	2.56^{b}	0.04	2.58^{b}	0.03
18:1n-11	1.91^{a}	0.04	1.72^{b}	0.04	1.74^{b}	0.02	1.73^{b}	0.02
18:1n-9	17.23	0.51	16.27	0.73	16.32	0.22	16.28	0.49
18:1n-7	3.85^{a}	0.13	3.54^{ab}	0.11	3.49^{b}	0.15	3.51^{b}	0.12
18:2n-6	3.32	0.04	3.16	0.10	3.22	0.07	3.19	0.05
18:3n-6	0.66	0.02	0.61	0.01	0.64	0.01	0.66	0.04
18:3n-3	0.97^{a}	0.01	1.07^{b}	0.02	1.13^{c}	0.01	1.21^{d}	0.03
18:4n-6	1.89^{a}	0.03	2.14^{b}	0.05	2.13^{b}	0.04	2.27^{c}	0.04
18:4n-3	0.13^{a}	0.01	0.20^{b}	0.01	0.10^{c}	0.00	0.11^{ac}	0.01
20:0	1.20^{a}	0.03	1.05^{b}	0.01	1.11^{ab}	0.05	1.09^{ab}	0.06
20:1n-11	0.77^{a}	0.05	0.55^{b}	0.02	0.60^{b}	0.05	0.59^{b}	0.05
20:1n-9	2.87^{a}	0.08	2.45^{b}	0.11	2.52^{b}	0.08	2.51^{b}	0.06
20:2n-6	0.39^{a}	0.00	0.37^{b}	0.01	0.38^{ab}	0.00	0.39^{a}	0.01
20:4n-6	1.33	0.09	1.33	0.06	1.35	0.05	1.46	0.09
20:4n-3	1.57^{ab}	0.04	1.49^{a}	0.06	1.60^{ab}	0.05	1.68^{b}	0.08
20:5n-3	7.04^{a}	0.18	7.63^{b}	0.15	7.40^{ab}	0.18	7.71^{b}	0.21
22:1n-13	0.15^{a}	0.01	-	-	0.18^{b}	0.00	0.18^{c}	0.01
22:1n-11	0.71^{a}	0.01	0.57^{b}	0.01	0.59^{b}	0.01	0.58^{b}	0.03
22:2n-6	0.54^{a}	0.05	0.78^{b}	0.04	0.48^{a}	0.00	0.49^{a}	0.01
22:4n-6	0.31^{a}	0.01	0.29^{ab}	0.01	0.28^{b}	0.00	0.28^{b}	0.01
22:4n-3	0.39	0.03	0.37	0.05	0.42	0.01	0.41	0.05
22:5n-6	0.32^{ab}	0.01	0.46^{a}	0.14	0.28^{ab}	0.03	0.19^{b}	0.02
22:5n-3	1.58	0.14	1.63	0.23	1.68	0.09	1.77	0.11
22:6n-3	7.34	0.24	7.42	0.15	7.48	0.29	7.52	0.35
24:1	0.20^{a}	0.02	-	-	0.14^{b}	0.02	0.13^{b}	0.00
DHA/EPA	1.04^{a}	0.01	0.97^{b}	0.03	1.01^{ab}	0.03	0.98^{b}	0.01
EPA/AA	5.29^{a}	0.03	5.75^{b}	0.03	5.50^{c}	0.02	5.29^{a}	0.03
DHA/AA	5.52^{a}	0.05	5.59^{a}	0.12	5.56^{a}	0.14	5.16^{b}	0.00

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. Standard deviation (SD) values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Appendix 5.6. Fatty acid composition (%) of total lipids of rotifers (*Brachionus plicatilis*) enriched for 12 hours with menhaden oil emulsion at different concentrations.

Fatty acid	Emulsion concentrations (g/million rotifers)							
	0.2		0.4		0.6		0.8	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
12:0	0.10	0.01	0.10	0.01	0.10	0.01	0.10	0.01
14:0	3.98^{a}	0.09	4.44^{b}	0.07	4.99^{c}	0.01	6.21^{d}	0.09
14:1n-5	0.63^{a}	0.01	0.65^{b}	0.00	0.65^{b}	0.00	0.52^{c}	0.00
15:0	0.48^{a}	0.00	0.47^{a}	0.00	0.51^{b}	0.00	0.60^{c}	0.00
16:0	9.78^{a}	0.17	10.42^{a}	0.30	13.13^{b}	0.20	18.32^{c}	0.33
16:1n-7	15.22^{a}	0.27	15.37^{a}	0.16	14.37^{b}	0.20	12.23^{c}	0.17
16:2n-4	0.47^{ab}	0.03	0.46^{a}	0.01	0.47^{ab}	0.01	0.49^{b}	0.01
16:3n-4	0.42^{a}	0.01	0.54^{b}	0.01	0.51^{c}	0.00	0.45^{d}	0.01
16:4n-3	1.39^{a}	0.05	1.68^{b}	0.07	1.70^{b}	0.07	1.74^{b}	0.05
17:0	0.25^{a}	0.01	0.26^{a}	0.01	0.32^{b}	0.02	0.44^{c}	0.03
17:1	1.54^{ac}	0.03	1.67^{b}	0.02	1.51^{a}	0.07	1.41^{c}	0.02
18:0	2.45^{a}	0.05	2.52^{a}	0.02	2.99^{b}	0.09	3.94^{c}	0.14
18:1n-11	1.36^{a}	0.04	1.09^{b}	0.02	0.89^{c}	0.00	-	-
18:1n-9	16.21^{a}	0.29	14.88^{b}	0.11	14.43^{b}	0.36	14.58^{b}	0.40
18:1n-7	3.69	0.11	3.62	0.07	3.84	0.05	3.62	0.13
18:2n-6	2.70^{a}	0.05	2.84^{b}	0.03	2.76^{a}	0.01	2.38^{c}	0.01
18:3n-6	0.54	0.02	0.56	0.01	0.55	0.01	0.37	0.20
18:3n-3	1.09^{a}	0.02	1.22^{b}	0.02	1.19^{b}	0.01	1.03^{c}	0.02
18:4n-6	2.15^{a}	0.07	2.55^{b}	0.07	2.42^{b}	0.06	2.14^{a}	0.08
18:4n-3	0.18^{a}	0.02	0.18^{a}	0.03	0.14^{b}	0.00	0.19^{a}	0.00
20:0	1.01^{a}	0.07	0.95^{ab}	0.07	0.79^{b}	0.04	0.61^{c}	0.06
20:1n-11	0.55^{a}	0.00	0.55^{a}	0.05	0.51^{a}	0.00	0.38^{b}	0.03
20:1n-9	2.34	0.06	2.27	0.08	2.28	0.02	2.19	0.04
20:2n-6	0.40^{a}	0.00	0.38^{b}	0.01	0.35^{c}	0.00	0.29^{d}	0.00
20:4n-6	1.37^{a}	0.01	1.23^{b}	0.04	1.15^{c}	0.03	0.95^{d}	0.01
20:4n-3	1.85^{a}	0.02	1.96^{b}	0.03	1.78^{c}	0.02	1.44^{d}	0.02
20:5n-3	9.57^{a}	0.10	9.16^{a}	0.24	8.49^{b}	0.30	7.70^{c}	0.14
22:0	-	-	-	-	-	-	0.10^{a}	0.01
22:1n-13	-	-	0.13^{a}	0.01	0.12^{ab}	0.00	0.11^{b}	0.00
22:1n-11	0.59^{a}	0.01	0.54^{b}	0.01	0.50^{c}	0.01	0.40^{d}	0.00
22:2n-6	0.85^{a}	0.18	0.57^{b}	0.01	0.54^{b}	0.00	0.52^{b}	0.01
22:4n-6	0.29^{a}	0.05	0.25^{ab}	0.02	0.27^{ab}	0.02	0.21^{b}	0.01
22:4n-3	0.46	0.13	0.40	0.12	0.42	0.00	0.36	0.01
22:5n-6	0.23^{a}	0.03	0.16^{bc}	0.00	0.20^{ac}	0.02	0.13^{b}	0.01
22:5n-3	2.51^{a}	0.04	2.27^{b}	0.03	1.99^{c}	0.12	1.82^{c}	0.04
22:6n-3	9.27^{a}	0.29	8.81^{ab}	0.35	8.24^{bc}	0.41	7.41^{c}	0.24
24:1	-	-	-	-	0.10^{a}	0.00	-	-
DHA/EPA	0.97	0.00	0.96	0.00	0.97	0.01	0.96	0.05
EPA/AA	6.98^{a}	0.07	7.46^{a}	0.28	7.41^{a}	0.24	8.11^{b}	0.26
DHA/AA	6.77^{a}	0.06	7.18^{a}	0.28	7.18^{a}	0.35	7.81^{b}	0.14

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. Standard deviation (SD) values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Appendix 5.7. Fatty acid composition (%) of total lipids of rotifers (*Brachionus plicatilis*) enriched for 18 hours with menhaden oil at different concentrations.

Fatty acid	Emulsion concentrations (g/million rotifers)							
	0.2		0.4		0.6		0.8	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
12:0	0.10^{a}	0.00	0.10^{a}	0.00	0.12^{b}	0.01	0.12^{b}	0.00
14:0	3.94^{a}	0.03	4.59^{b}	0.06	5.23^{c}	0.07	6.20^{d}	0.07
14:1n-5	0.70^{a}	0.00	0.69^{a}	0.01	0.81^{b}	0.01	0.69^{a}	0.01
15:0	0.49^{a}	0.00	0.53^{b}	0.01	0.58^{c}	0.01	0.66^{d}	0.00
16:0	12.37^{a}	0.34	14.61^{b}	0.50	16.19^{c}	0.25	20.06^{d}	0.23
16:1n-7	13.62^{a}	0.11	13.38^{a}	0.18	14.44^{b}	0.21	13.34^{a}	0.15
16:2n-4	0.56^{a}	0.00	0.56^{a}	0.01	0.57^{ab}	0.01	0.59^{b}	0.01
16:3n-4	0.32^{a}	0.00	0.33^{a}	0.00	0.35^{ab}	0.01	0.41^{b}	0.05
16:4n-3	1.21^{a}	0.03	1.31^{b}	0.02	1.33^{b}	0.02	1.43^{c}	0.01
17:0	0.33^{a}	0.00	0.40^{b}	0.01	0.41^{b}	0.01	0.51^{c}	0.01
17:1	0.97^{a}	0.01	0.98^{a}	0.01	1.06^{b}	0.03	1.01^{a}	0.01
18:0	3.28^{a}	0.11	3.93^{b}	0.05	4.19^{c}	0.07	4.78^{d}	0.05
18:1n-11	1.35^{a}	0.01	1.13^{b}	0.01	1.35^{a}	0.02	0.90^{c}	0.02
18:1n-9	16.04^{a}	0.37	15.84^{a}	0.21	17.06^{b}	0.25	16.27^{a}	0.16
18:1n-7	4.38^{a}	0.05	4.68^{b}	0.07	5.22^{c}	0.09	4.83^{b}	0.07
18:2n-6	2.45^{a}	0.01	2.50^{a}	0.03	2.69^{b}	0.04	2.37^{c}	0.02
18:3n-6	0.61^{a}	0.04	0.60^{a}	0.00	0.59^{a}	0.03	0.52^{b}	0.00
18:3n-3	0.91^{a}	0.01	0.95^{b}	0.01	0.89^{a}	0.01	0.94^{b}	0.01
18:4n-6	1.60^{a}	0.01	1.56^{b}	0.02	1.46^{c}	0.00	1.25^{d}	0.01
18:4n-3	0.13^{a}	0.00	0.17^{b}	0.00	0.18^{b}	0.00	0.22^{c}	0.00
20:0	1.02^{a}	0.06	0.90^{b}	0.04	0.86^{b}	0.01	0.72^{c}	0.01
20:1n-11	0.56	0.04	0.55	0.03	0.52	0.01	0.51	0.01
20:1n-9	2.57^{a}	0.04	2.62^{a}	0.00	2.60^{a}	0.06	2.74^{b}	0.03
20:2n-6	0.38^{a}	0.01	0.36^{ab}	0.01	0.34^{b}	0.01	0.29^{c}	0.01
20:4n-6	1.39^{a}	0.07	1.34^{a}	0.02	1.19^{b}	0.01	0.95^{c}	0.01
20:4n-3	1.64^{a}	0.00	1.56^{b}	0.02	1.41^{c}	0.00	1.16^{d}	0.01
20:5n-3	8.43^{a}	0.14	7.28^{b}	0.18	5.97^{c}	0.18	4.96^{d}	0.14
22:0	-	-	-	-	-	-	0.12^{a}	0.01
22:1n-13	0.15	0.02	0.15	0.00	0.13	0.01	0.15	0.00
22:1n-11	0.65^{a}	0.01	0.61^{b}	0.01	0.68^{c}	0.00	0.59^{d}	0.00
22:2n-6	0.58^{a}	0.01	0.51^{ab}	0.01	0.50^{ab}	0.13	0.37^{b}	0.01
22:4n-6	0.28^{a}	0.02	0.26^{a}	0.01	0.20^{b}	0.02	0.17^{b}	0.01
22:4n-3	0.38^{a}	0.02	0.36^{ac}	0.02	0.21^{b}	0.09	0.24^{bc}	0.03
22:5n-6	0.39^{a}	0.03	0.30^{b}	0.01	0.27^{b}	0.02	0.25^{b}	0.04
22:5n-3	2.50^{a}	0.33	2.10^{a}	0.23	1.45^{b}	0.08	1.24^{b}	0.17
22:6n-3	8.28^{a}	0.24	7.21^{b}	0.14	5.55^{c}	0.13	4.58^{d}	0.14
24:1	0.10^{a}	0.01	0.10^{a}	0.01	0.19^{b}	0.04	0.23^{b}	0.01
DHA/EPA	0.98^{a}	0.01	0.99^{a}	0.01	0.93^{b}	0.01	0.92^{b}	0.00
EPA/AA	6.08^{a}	0.34	5.45^{b}	0.00	5.03^{b}	0.03	5.22^{b}	0.02
DHA/AA	5.97^{a}	0.27	5.39^{b}	0.04	4.68^{c}	0.01	4.82^{c}	0.01

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. Standard deviation (SD) values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Appendix 5.8. Fatty acid composition (%) of total lipids of rotifers (*Brachionus plicatilis*) enriched for 24 hours with menhaden oil emulsion at different concentrations.

Fatty acid	Emulsion concentrations (g/million rotifers)							
	0.2		0.4		0.6		0.8	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
12:0	0.12	0.00	0.11	0.00	0.12	0.02	0.12	0.02
14:0	3.75^{a}	0.01	4.14^{b}	0.08	5.00^{c}	0.05	6.71^{d}	0.07
14:1n-5	0.73^{a}	0.00	0.71^{a}	0.00	0.81^{b}	0.02	0.70^{a}	0.02
15:0	0.50^{a}	0.01	0.50^{a}	0.00	0.60^{b}	0.03	0.78^{c}	0.05
16:0	11.63^{a}	0.25	11.09^{a}	0.18	17.07^{b}	0.19	23.65^{c}	0.31
16:1n-7	14.01^{ab}	0.56	14.84^{a}	0.31	13.69^{b}	0.17	11.21^{c}	0.10
16:2n-4	0.54^{ab}	0.01	0.51^{a}	0.00	0.57^{bc}	0.02	0.62^{c}	0.04
16:3n-4	0.32^{a}	0.01	0.33^{a}	0.00	0.36^{a}	0.02	0.44^{b}	0.05
16:4n-3	1.15^{a}	0.02	1.30^{b}	0.02	1.31^{b}	0.01	1.41^{c}	0.03
17:0	0.32^{a}	0.01	0.28^{a}	0.00	0.49^{b}	0.01	0.65^{c}	0.03
17:1	0.94^{a}	0.02	1.05^{b}	0.01	0.87^{c}	0.02	1.00^{b}	0.03
18:0	3.23^{a}	0.03	2.73^{b}	0.04	4.74^{c}	0.03	5.53^{d}	0.04
18:1n-11	1.45^{a}	0.01	1.26^{b}	0.03	0.92^{c}	0.05	0.50^{d}	0.01
18:1n-9	16.14^{a}	0.37	16.19^{a}	0.29	14.28^{b}	0.45	13.93^{b}	0.22
18:1n-7	4.79^{a}	0.11	4.83^{a}	0.13	5.56^{b}	0.16	5.08^{c}	0.07
18:2n-6	2.23^{a}	0.01	2.45^{b}	0.03	2.03^{c}	0.01	1.48^{d}	0.01
18:3n-6	0.57^{a}	0.02	0.54^{a}	0.02	0.54^{a}	0.01	0.44^{b}	0.01
18:3n-3	0.90^{a}	0.01	1.04^{b}	0.01	0.89^{a}	0.01	0.67^{c}	0.01
18:4n-6	1.47^{a}	0.01	1.67^{b}	0.02	1.39^{c}	0.01	1.19^{d}	0.02
18:4n-3	0.15^{a}	0.01	0.12^{b}	0.00	0.24^{c}	0.00	0.29^{d}	0.01
20:0	0.90^{a}	0.00	0.89^{a}	0.01	0.69^{b}	0.02	0.48^{c}	0.01
20:1n-11	0.62^{a}	0.01	0.57^{a}	0.02	0.57^{a}	0.04	0.45^{b}	0.01
20:1n-9	2.84^{a}	0.00	2.65^{b}	0.03	2.78^{ac}	0.02	2.75^{c}	0.05
20:2n-6	0.37^{a}	0.01	0.38^{a}	0.00	0.30^{b}	0.00	0.22^{c}	0.00
20:4n-6	1.26^{a}	0.01	1.17^{b}	0.03	0.95^{c}	0.01	0.63^{d}	0.01
20:4n-3	1.64^{a}	0.01	1.85^{b}	0.03	1.42^{c}	0.01	0.89^{d}	0.01
20:5n-3	8.18^{a}	0.24	8.38^{a}	0.16	6.19^{b}	0.09	5.27^{c}	0.19
22:0	-	-	-	-	0.12^{a}	0.00	0.18^{b}	0.01
22:1n-13	0.17^{a}	0.00	0.13^{b}	0.01	0.15^{c}	0.01	0.14^{bc}	0.00
22:1n-11	0.73^{a}	0.01	0.65^{b}	0.01	0.63^{c}	0.00	0.51^{d}	0.00
22:2n-6	0.57^{a}	0.01	0.58^{a}	0.01	0.44^{b}	0.01	0.38^{c}	0.00
22:4n-6	0.28^{a}	0.01	0.27^{a}	0.03	0.24^{ab}	0.05	0.19^{b}	0.01
22:4n-3	0.48^{a}	0.01	0.43^{b}	0.02	0.29^{c}	0.02	0.26^{c}	0.01
22:5n-6	0.32^{a}	0.05	0.26^{ab}	0.04	0.19^{b}	0.07	0.14^{b}	0.01
22:5n-3	2.59^{a}	0.01	2.56^{a}	0.01	1.71^{b}	0.01	1.26^{c}	0.02
22:6n-3	8.46^{a}	0.33	8.53^{a}	0.27	6.24^{b}	0.16	5.28^{c}	0.19
24:1	-	-	-	-	0.17^{a}	0.00	0.14^{b}	0.00
DHA/EPA	1.04	0.03	1.02	0.01	1.01	0.01	1.00	0.01
EPA/AA	6.50^{a}	0.11	7.16^{b}	0.32	6.54^{a}	0.15	8.39^{c}	0.15
DHA/AA	6.73^{a}	0.20	7.29^{b}	0.25	6.60^{a}	0.13	8.40^{c}	0.15

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. Standard deviation (SD) values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Appendix 5.9. Fatty acid composition (%) of total lipids of rotifers (*Brachionus plicatilis*) enriched for 6 hours with Algamac-3010 emulsion at different concentrations.

Fatty acid	Emulsion concentrations (g/million rotifers)							
	0.2		0.3		0.4		0.5	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
12:0	0.20^{a}	0.00	0.22^{b}	0.00	0.23^{b}	0.00	0.23^{b}	0.00
14:0	5.30^{a}	0.14	7.10^{b}	0.10	7.07^{b}	0.09	7.70^{c}	0.12
14:1n-5	0.45	0.02	0.54	0.05	0.51	0.07	0.49	0.04
15:0	0.41^{a}	0.02	0.54^{b}	0.00	0.52^{b}	0.00	0.53^{b}	0.00
16:0	12.90^{a}	0.36	17.47^{b}	0.24	16.69^{c}	0.21	19.29^{d}	0.25
16:1n-7	10.37^{a}	0.28	9.87^{ab}	0.16	9.44^{b}	0.24	8.70^{c}	0.13
16:2n-4	0.26	0.01	0.27	0.04	0.28	0.00	0.24	0.00
16:3n-4	0.18^{a}	0.00	0.23^{b}	0.03	0.16^{c}	0.00	0.13^{d}	0.00
16:4n-3	0.11^{a}	0.00	0.17^{b}	0.02	0.11^{a}	0.00	0.10^{a}	0.00
17:0	0.26^{a}	0.01	0.35^{b}	0.02	0.29^{a}	0.03	0.26^{a}	0.03
17:1	0.41^{a}	0.02	0.24^{b}	0.03	0.25^{b}	0.03	0.15^{c}	0.02
18:0	2.47^{a}	0.07	2.29^{b}	0.09	2.16^{b}	0.05	2.21^{b}	0.05
18:1n-11	1.94^{a}	0.05	1.45^{b}	0.01	1.40^{bc}	0.06	1.33^{c}	0.03
18:1n-9	10.51^{a}	0.29	8.55^{b}	0.12	8.50^{b}	0.17	8.16^{b}	0.11
18:1n-7	3.59^{a}	0.07	3.40^{b}	0.01	3.16^{c}	0.03	3.07^{c}	0.09
18:2n-6	2.88^{a}	0.03	2.43^{b}	0.07	2.36^{b}	0.05	2.21^{c}	0.03
18:3n-6	0.48^{a}	0.02	0.46^{a}	0.01	0.45^{a}	0.03	0.68^{b}	0.08
18:3n-3	0.15^{a}	0.03	0.25^{b}	0.03	0.15^{a}	0.02	0.24^{b}	0.02
18:4n-6	0.21^{a}	0.00	0.21^{b}	0.00	0.21^{a}	0.01	0.28^{b}	0.02
18:4n-3	0.10	0.00	-	-	0.10	0.00	0.11	0.00
20:0	1.00^{a}	0.02	0.63^{b}	0.00	0.81^{ab}	0.22	0.68^{b}	0.09
20:1n-11	0.59^{a}	0.02	0.43^{b}	0.01	0.41^{b}	0.04	0.40^{b}	0.01
20:1n-9	1.85^{a}	0.06	1.28^{b}	0.01	1.37^{b}	0.10	1.31^{b}	0.05
20:2n-6	0.53^{a}	0.02	0.49^{ab}	0.01	0.51^{ab}	0.01	0.47^{b}	0.02
20:4n-6	2.07^{a}	0.01	1.79^{b}	0.03	1.76^{b}	0.01	1.71^{b}	0.06
20:4n-3	0.87^{a}	0.03	0.87^{a}	0.01	0.90^{a}	0.05	1.42^{b}	0.09
20:5n-3	3.92^{a}	0.12	3.24^{a}	0.05	3.23^{b}	0.04	3.01^{c}	0.06
22:1n-13	0.22^{a}	0.01	-	-	0.19^{a}	0.00	0.13^{c}	0.04
22:1n-11	0.70^{a}	0.02	0.48^{b}	0.02	0.47^{b}	0.01	0.42^{c}	0.01
22:2n-6	0.44	0.02	0.40	0.01	0.41	0.01	0.42	0.02
22:4n-6	0.45	0.05	0.38	0.02	0.38	0.04	0.37	0.03
22:4n-3	1.12^{a}	0.08	0.69^{b}	0.03	0.23^{c}	0.03	0.92^{d}	0.10
22:5n-6	0.36^{a}	0.02	0.36^{a}	0.04	0.46^{b}	0.04	0.19^{c}	0.03
22:5n-3	1.40^{a}	0.01	0.70^{b}	0.01	0.89^{c}	0.02	0.89^{c}	0.02
22:6n-3	24.59^{a}	0.73	25.41^{ab}	0.51	25.91^{ab}	0.57	26.76^{b}	0.28
24:1	0.19^{a}	0.01	-	-	0.16^{a}	0.00	0.22^{b}	0.03
DHA/EPA	6.27^{a}	0.10	7.85^{b}	0.14	8.03^{b}	0.18	8.88^{c}	0.27
EPA/AA	1.90	0.06	1.81	0.02	1.84	0.02	1.76	0.10
DHA/AA	11.90^{a}	0.37	14.20^{b}	0.22	14.76^{bc}	0.33	15.64^{c}	0.42

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. Standard deviation (SD) values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Appendix 5.10. Fatty acid composition (%) of total lipids of rotifers (*Brachionus plicatilis*) enriched for 12 hours with Algamac-3010 emulsion at different concentrations.

Fatty acid	Emulsion concentrations (g/million rotifers)							
	0.2		0.3		0.4		0.5	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
12:0	0.21^{a}	0.01	0.23^{b}	0.00	0.23^{b}	0.00	0.23^{b}	0.00
14:0	5.48^{a}	0.31	7.29^{b}	0.33	7.09^{b}	0.25	7.66^{b}	0.34
14:1n-5	0.46^{a}	0.03	0.55^{b}	0.00	0.51^{ab}	0.00	0.49^{ab}	0.05
15:0	0.43^{a}	0.03	0.55^{b}	0.00	0.52^{b}	0.01	0.53^{b}	0.02
16:0	13.34^{a}	0.76	17.93^{bc}	0.88	16.96^{b}	0.43	19.18^{c}	0.79
16:1n-7	10.72^{a}	0.60	10.06^{ab}	0.34	9.53^{bc}	0.27	8.65^{c}	0.37
16:2n-4	0.27	0.01	0.27	0.04	0.28	0.00	0.24	0.01
16:3n-4	0.18^{a}	0.01	0.23^{b}	0.02	0.16^{a}	0.01	0.12^{c}	0.00
16:4n-3	0.11^{a}	0.00	0.17^{b}	0.02	0.11^{a}	0.00	0.08^{c}	0.00
17:0	0.26^{a}	0.01	0.36^{b}	0.01	0.30^{a}	0.01	0.25^{a}	0.04
17:1	0.42^{a}	0.01	0.25^{b}	0.03	0.25^{b}	0.00	0.14^{c}	0.01
18:0	2.55^{a}	0.14	2.33^{ab}	0.03	2.18^{b}	0.01	2.20^{b}	0.11
18:1n-11	2.00^{a}	0.11	1.48^{b}	0.01	1.41^{b}	0.01	1.32^{b}	0.06
18:1n-9	10.86^{a}	0.61	8.78^{b}	0.54	8.58^{b}	0.65	8.11^{b}	0.33
18:1n-7	3.71^{a}	0.18	3.47^{ab}	0.02	3.19^{bc}	0.04	3.05^{c}	0.17
18:2n-6	2.97^{a}	0.11	2.48^{b}	0.01	2.39^{b}	0.01	2.20^{c}	0.09
18:3n-6	0.49^{a}	0.04	0.47^{a}	0.01	0.45^{a}	0.00	0.67^{b}	0.04
18:3n-3	0.15^{a}	0.01	0.26^{b}	0.00	0.15^{a}	0.02	0.24^{b}	0.01
18:4n-6	0.21^{a}	0.03	0.21^{a}	0.00	0.22^{a}	0.00	0.28^{b}	0.03
18:4n-3	0.10	0.01	-	-	0.10	0.00	0.11	0.01
20:0	1.03^{a}	0.05	0.64^{b}	0.01	0.82^{c}	0.04	0.67^{b}	0.05
20:1n-11	0.61^{a}	0.04	0.43^{b}	0.00	0.41^{b}	0.04	0.40^{b}	0.02
20:1n-9	1.91^{a}	0.11	1.30^{b}	0.03	1.38^{b}	0.10	1.30^{b}	0.10
20:2n-6	0.55^{a}	0.03	0.50^{ab}	0.03	0.51^{ab}	0.01	0.47^{b}	0.03
20:4n-6	2.19^{a}	0.14	1.80^{b}	0.10	1.77^{b}	0.09	1.69^{b}	0.08
20:4n-3	0.90^{a}	0.06	0.88^{a}	0.00	0.91^{a}	0.00	1.40^{b}	0.08
20:5n-3	4.05^{a}	0.24	3.28^{b}	0.11	3.24^{b}	0.12	2.98^{b}	0.11
22:1n-13	0.23^{a}	0.02	-	-	0.19^{ab}	0.01	0.13^{b}	0.00
22:1n-11	0.72^{a}	0.04	0.49^{b}	0.02	0.47^{bc}	0.01	0.42^{c}	0.02
22:2n-6	0.45	0.03	0.41	0.04	0.41	0.02	0.42	0.03
22:4n-6	0.46^{a}	0.02	0.38^{b}	0.03	0.38^{b}	0.03	0.37^{b}	0.04
22:4n-3	1.16^{a}	0.03	0.70^{b}	0.05	0.24^{c}	0.02	0.92^{d}	0.05
22:5n-6	0.37^{a}	0.01	0.36^{a}	0.03	0.47^{b}	0.04	0.19^{c}	0.03
22:5n-3	1.44^{a}	0.01	0.71^{b}	0.01	0.90^{c}	0.02	0.89^{c}	0.04
22:6n-3	26.71	1.58	26.25	0.19	26.55	0.13	26.24	1.01
24:1	0.20^{ab}	0.03	-	-	0.17^{a}	0.00	0.22^{b}	0.03
DHA/EPA	6.59^{a}	0.01	8.01^{b}	0.03	8.19^{c}	0.00	8.82^{d}	0.01
EPA/AA	1.85	0.01	1.82	0.00	1.83	0.00	1.76	0.07
DHA/AA	12.19^{a}	0.04	14.57^{b}	0.03	14.97^{bc}	0.03	15.53^{c}	0.57

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. Standard deviation (SD) values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Appendix 5.11. Fatty acid composition (%) of total lipids of rotifers (*Brachionus plicatilis*) enriched for 18 hours with Algamac-3010 emulsion at different concentrations.

Fatty acid	Emulsion concentrations (g/million rotifers)							
	0.2		0.3		0.4		0.5	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
12:0	0.23	0.00	0.23	0.00	0.22	0.01	0.23	0.00
14:0	6.98^{a}	0.04	7.24^{b}	0.05	6.98^{a}	0.02	7.54^{c}	0.11
14:1n-5	0.51^{a}	0.00	0.54^{b}	0.00	0.51^{a}	0.00	0.45^{c}	0.01
15:0	0.54^{a}	0.00	0.54^{a}	0.00	0.51^{b}	0.00	0.52^{b}	0.01
16:0	18.09^{a}	0.12	17.71^{a}	0.19	16.74^{b}	0.24	19.01^{c}	0.30
16:1n-7	9.15^{a}	0.06	9.80^{b}	0.08	9.35^{a}	0.02	8.47^{c}	0.13
16:2n-4	0.28^{a}	0.01	0.29^{a}	0.01	0.28^{a}	0.00	0.24^{b}	0.00
16:3n-4	0.16^{a}	0.01	0.17^{a}	0.00	0.16^{a}	0.00	0.14^{b}	0.00
16:4n-3	0.10^{a}	0.00	0.19^{b}	0.01	0.10^{a}	0.00	0.10^{a}	0.00
17:0	0.34	0.00	0.29	0.03	0.31	0.03	0.31	0.01
17:1	0.40^{a}	0.02	0.82^{b}	0.01	0.44^{c}	0.00	0.22^{d}	0.00
18:0	2.50^{a}	0.02	2.27^{b}	0.03	2.18^{c}	0.01	2.11^{c}	0.04
18:1n-11	1.39^{a}	0.01	1.45^{a}	0.04	1.39^{a}	0.02	1.28^{b}	0.02
18:1n-9	8.33	0.37	8.53	0.23	8.49	0.41	7.86	0.13
18:1n-7	3.01^{a}	0.02	3.36^{b}	0.03	3.15^{c}	0.04	2.94^{a}	0.05
18:2n-6	2.34^{a}	0.02	2.42^{a}	0.04	2.35^{a}	0.06	2.14^{b}	0.04
18:3n-6	0.46^{a}	0.00	0.49^{b}	0.00	0.47^{c}	0.00	0.40^{d}	0.00
18:3n-3	0.14	0.02	0.10	0.02	0.12	0.01	0.12	0.00
18:4n-6	0.19^{a}	0.00	0.21^{ab}	0.00	0.22^{b}	0.01	0.21^{ab}	0.01
18:4n-3	0.11^{a}	0.00	0.19^{b}	0.00	0.10^{a}	0.01	0.10^{a}	0.00
20:0	0.69^{a}	0.01	0.65^{b}	0.01	0.69^{a}	0.01	0.62^{c}	0.01
20:1n-11	0.45^{ab}	0.00	0.45^{ab}	0.03	0.47^{a}	0.02	0.41^{b}	0.00
20:1n-9	1.41^{a}	0.06	1.30^{b}	0.02	1.58^{c}	0.02	1.41^{a}	0.02
20:2n-6	0.49^{ab}	0.00	0.49^{ab}	0.00	0.51^{a}	0.01	0.48^{b}	0.00
20:4n-6	1.81	0.04	1.76	0.01	1.75	0.03	1.65	0.14
20:4n-3	0.84^{a}	0.01	0.86^{a}	0.01	0.90^{b}	0.00	0.84^{a}	0.01
20:5n-3	3.10^{a}	0.02	3.17^{b}	0.02	3.19^{b}	0.01	2.99^{c}	0.04
22:1n-13	0.17	0.00	0.18	0.01	0.18	0.00	0.17	0.00
22:1n-11	0.46^{a}	0.01	0.47^{a}	0.00	0.46^{a}	0.00	0.42^{b}	0.01
22:2n-6	0.43^{a}	0.03	0.41^{a}	0.01	0.42^{a}	0.00	0.50^{b}	0.03
22:4n-6	0.37^{a}	0.02	0.39^{a}	0.01	0.34^{b}	0.00	0.39^{a}	0.02
22:4n-3	0.94^{a}	0.07	0.42^{b}	0.04	0.32^{b}	0.01	0.34^{b}	0.02
22:5n-6	0.32^{a}	0.03	0.50^{bc}	0.05	0.40^{ab}	0.05	0.57^{c}	0.04
22:5n-3	0.82^{a}	0.02	0.77^{a}	0.03	0.87^{a}	0.03	1.01^{b}	0.08
22:6n-3	25.76	0.27	25.20	0.44	26.16	0.52	25.84	0.23
24:1	0.19^{a}	0.01	0.18^{a}	0.02	0.17^{a}	0.02	0.27^{b}	0.03
DHA/EPA	8.30^{a}	0.03	7.96^{b}	0.05	8.21^{a}	0.03	8.64^{c}	0.05
EPA/AA	1.72	0.05	1.80	0.03	1.82	0.04	1.82	0.13
DHA/AA	14.27	0.46	14.29	0.12	14.97	0.29	15.75	1.18

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. Standard deviation (SD) values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Appendix 5.12. Fatty acid composition (%) of total lipids of rotifers (*Brachionus plicatilis*) enriched for 24 hours with Algamac-3010 emulsion at different concentrations.

Fatty acid	Emulsion concentrations (g/million rotifers)							
	0.2		0.3		0.4		0.5	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
12:0	0.23	0.00	0.23	0.00	0.22	0.01	0.23	0.00
14:0	6.98^{a}	0.08	7.29^{a}	0.25	7.03^{a}	0.16	7.63^{b}	0.02
14:1n-5	0.51^{a}	0.01	0.55^{a}	0.02	0.51^{a}	0.01	0.46^{b}	0.00
15:0	0.54	0.01	0.54	0.02	0.52	0.01	0.53	0.00
16:0	17.98^{a}	0.35	17.84^{ab}	0.58	16.85^{b}	0.40	18.67^{c}	0.14
16:1n-7	9.15^{a}	0.10	9.87^{b}	0.35	9.41^{a}	0.22	8.47^{c}	0.13
16:2n-4	0.28^{a}	0.00	0.29^{a}	0.01	0.28^{a}	0.01	0.25^{b}	0.00
16:3n-4	0.16	0.01	0.17	0.01	0.16	0.00	0.15	0.00
16:4n-3	0.10^{a}	0.00	0.19^{b}	0.02	0.10^{a}	0.00	0.10^{a}	0.00
17:0	0.34	0.00	0.29	0.03	0.32	0.04	0.31	0.00
17:1	0.40^{a}	0.01	0.82^{b}	0.03	0.45^{c}	0.01	0.22^{d}	0.00
18:0	2.50^{a}	0.02	2.29^{b}	0.06	2.19^{bc}	0.05	2.13^{c}	0.06
18:1n-11	1.39^{a}	0.01	1.46^{a}	0.03	1.40^{a}	0.03	1.29^{b}	0.05
18:1n-9	8.28^{a}	0.14	8.60^{a}	0.27	8.54^{a}	0.21	7.96^{b}	0.13
18:1n-7	3.01^{a}	0.03	3.39^{b}	0.12	3.17^{bc}	0.08	2.97^{ac}	0.12
18:2n-6	2.34	0.02	2.44	0.07	2.36	0.06	2.17	0.08
18:3n-6	0.46^{a}	0.00	0.49^{b}	0.01	0.47^{ab}	0.01	0.40^{c}	0.00
18:3n-3	0.14^{ab}	0.02	0.10^{a}	0.02	0.12^{a}	0.02	0.12^{b}	0.00
18:4n-6	0.19	0.01	0.21	0.01	0.22	0.00	0.21	0.01
18:4n-3	0.11^{a}	0.00	0.19^{b}	0.02	0.10^{a}	0.01	0.10^{a}	0.00
20:0	0.69^{a}	0.02	0.66^{ab}	0.07	0.70^{a}	0.02	0.63^{b}	0.02
20:1n-11	0.45^{a}	0.01	0.46^{a}	0.02	0.47^{a}	0.03	0.41^{b}	0.01
20:1n-9	1.41^{abc}	0.19	1.31^{a}	0.01	1.59^{b}	0.06	1.43^{c}	0.00
20:2n-6	0.49	0.00	0.49	0.01	0.51	0.01	0.48	0.00
20:4n-6	1.80	0.06	1.80	0.01	1.76	0.08	1.70	0.04
20:4n-3	0.84^{a}	0.01	0.87^{ab}	0.02	0.91^{b}	0.02	0.85^{a}	0.01
20:5n-3	3.10	0.03	3.13	0.02	3.21	0.07	3.09	0.09
22:1n-13	0.17	0.00	0.18	0.03	0.18	0.00	0.17	0.02
22:1n-11	0.46^{a}	0.00	0.47^{a}	0.01	0.46^{a}	0.01	0.42^{b}	0.02
22:2n-6	0.43^{a}	0.02	0.42^{a}	0.00	0.42^{a}	0.01	0.50^{b}	0.03
22:4n-6	0.37^{ab}	0.04	0.40^{a}	0.00	0.34^{b}	0.00	0.39^{a}	0.02
22:4n-3	0.94^{a}	0.05	0.42^{b}	0.04	0.32^{c}	0.01	0.34^{c}	0.03
22:5n-6	0.32^{a}	0.03	0.51^{bc}	0.04	0.40^{ab}	0.06	0.58^{c}	0.04
22:5n-3	0.82^{a}	0.00	0.77^{b}	0.03	0.87^{ab}	0.05	1.02^{c}	0.08
22:6n-3	25.57^{a}	0.44	25.85^{ab}	0.41	26.48^{bc}	0.02	26.95^{c}	0.43
24:1	0.19^{a}	0.02	0.18^{a}	0.03	0.17^{a}	0.01	0.27^{b}	0.03
DHA/EPA	8.24^{a}	0.23	8.27^{a}	0.19	8.26^{a}	0.19	8.73^{b}	0.12
EPA/AA	1.72	0.04	1.74	0.01	1.82	0.04	1.81	0.10
DHA/AA	14.20^{a}	0.75	14.38^{a}	0.27	15.06^{ab}	0.69	15.82^{b}	0.65

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. Standard deviation (SD) values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Appendix 5.13. Fatty acid composition (%) of total lipids of rotifers (*Brachionus plicatilis*) enriched for 6 hours with DHASCO emulsion at different concentrations.

Fatty acid	Emulsion concentration (g/million rotifers)							
	0.1		0.2		0.3		0.4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
12:0	0.70^{a}	0.03	0.91^{b}	0.10	0.95^{b}	0.03	1.28^{c}	0.02
14:0	5.90^{a}	0.28	7.06^{b}	0.32	7.50^{b}	0.33	7.63^{b}	0.42
14:1n-5	0.31^{a}	0.02	0.31^{a}	0.03	0.35^{ab}	0.00	0.40^{b}	0.02
15:0	0.18^{a}	0.00	0.16^{ab}	0.02	0.17^{ab}	0.01	0.14^{b}	0.01
16:0	6.14^{a}	0.32	6.65^{ab}	0.39	6.62^{ab}	0.13	7.03^{b}	0.18
16:1n-7	8.19^{a}	0.42	8.78^{ab}	0.38	9.24^{b}	0.44	9.72^{b}	0.27
16:2n-4	0.15	0.00	0.13	0.01	0.16	0.01	0.16	0.02
16:3n-4	0.10	0.03	0.10	0.01	0.10	0.01	-	-
17:0	0.15	0.02	0.15	0.02	0.16	0.01	0.14	0.01
17:1	0.28^{a}	0.01	0.27^{a}	0.03	0.31^{a}	0.00	0.22^{b}	0.02
18:0	1.78	0.10	1.79	0.18	1.94	0.10	2.01	0.09
18:1n-11	1.15^{a}	0.06	1.05^{a}	0.11	-	-	0.62^{b}	0.02
18:1n-9	29.68^{a}	0.59	29.44^{ab}	0.30	28.41^{b}	0.35	26.36^{c}	0.31
18:1n-7	1.17^{a}	0.06	1.98^{b}	0.08	2.10^{bc}	0.04	2.17^{c}	0.05
18:2n-6	1.94^{a}	0.12	2.54^{b}	0.22	2.43^{b}	0.12	2.38^{b}	0.08
18:3n-6	0.16	0.04	0.14	0.01	0.15	0.00	0.20	0.02
18:3n-3	0.11	0.03	0.10	0.01	0.11	0.01	0.12	0.00
18:4n-6	0.13^{a}	0.02	-	-	-	-	0.12^{a}	0.00
18:4n-3	-	-	-	-	-	-	0.10^{a}	0.00
20:0	0.70^{a}	0.01	0.69^{a}	0.06	0.86^{b}	0.01	0.72^{a}	0.07
20:1n-11	0.42	0.03	0.43	0.04	0.44	0.03	0.43	0.03
20:1n-9	1.92^{a}	0.07	2.10^{b}	0.03	2.24^{c}	0.01	2.27^{c}	0.06
20:2n-6	0.18^{a}	0.01	0.14^{b}	0.01	0.15^{b}	0.01	0.12^{c}	0.00
20:4n-6	0.37^{a}	0.02	0.46^{b}	0.02	0.47^{b}	0.00	0.50^{b}	0.02
20:4n-3	0.11^{a}	0.01	0.11^{a}	0.01	0.11^{a}	0.00	-	-
20:5n-3	1.64^{a}	0.10	1.84^{b}	0.10	1.90^{b}	0.04	1.95^{b}	0.04
22:1n-13	-	-	-	-	0.13^{a}	0.00	-	-
22:1n-11	0.51^{a}	0.04	0.51^{a}	0.05	0.61^{b}	0.01	0.48^{a}	0.02
22:2n-6	0.17	0.02	0.17	0.02	0.20	0.01	0.18	0.01
22:4n-6	-	-	0.24^{a}	0.01	0.12^{b}	0.01	0.10^{c}	0.00
22:4n-3	0.54^{a}	0.00	0.10^{b}	0.01	0.11^{b}	0.01	0.10^{b}	0.01
22:5n-6	0.21^{a}	0.01	0.22^{a}	0.01	0.16^{b}	0.01	0.23^{a}	0.01
22:5n-3	1.55^{a}	0.17	0.73^{b}	0.03	0.71^{b}	0.04	0.69^{b}	0.03
22:6n-3	25.38^{a}	0.79	27.12^{b}	0.25	28.00^{b}	0.50	28.38^{b}	0.37
24:1	-	-	0.12	0.01	0.10	0.02	0.13	0.01
DHA/EPA	15.51	1.32	14.76	0.66	14.75	0.56	14.54	0.47
EPA/AA	4.41	0.28	4.01	0.43	4.04	0.10	3.95	0.21
DHA/AA	68.24^{a}	1.53	59.08^{b}	3.67	59.53^{b}	0.79	57.36^{b}	1.18

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. Standard deviation (SD) values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHASCO, DHA-rich single cell oil; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Appendix 5.14. Fatty acid composition (%) of total lipids of rotifers (*Brachionus plicatilis*) enriched for 12 hours with DHASCO emulsion at different concentrations.

Fatty acid	Emulsion concentrations (g/million rotifers)							
	0.1		0.2		0.3		0.4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
12:0	0.79^{a}	0.01	0.94^{b}	0.04	0.94^{b}	0.01	1.77^{c}	0.04
14:0	6.63^{a}	0.13	7.33^{b}	0.27	7.53^{b}	0.35	9.91^{c}	0.23
14:1n-5	0.35^{a}	0.01	0.33^{a}	0.01	0.35^{a}	0.00	0.45^{b}	0.01
15:0	0.20^{a}	0.00	0.17^{b}	0.01	0.17^{b}	0.00	0.16^{b}	0.01
16:0	6.90^{a}	0.17	6.10^{b}	0.22	6.64^{ab}	0.25	9.22^{c}	0.20
16:1n-7	9.20^{a}	0.22	8.76^{a}	0.32	9.27^{a}	0.36	7.99^{b}	0.18
16:2n-4	0.17^{ac}	0.01	0.14^{b}	0.00	0.16^{a}	0.00	0.18^{c}	0.00
16:3n-4	0.10^{a}	0.02	0.11^{a}	0.00	0.10^{a}	0.01	-	-
17:0	0.17	0.02	0.15	0.01	0.16	0.01	0.15	0.00
17:1	0.31^{a}	0.00	0.28^{b}	0.01	0.31^{a}	0.00	0.24^{c}	0.00
18:0	2.00^{ac}	0.06	1.86^{b}	0.07	1.95^{ab}	0.01	2.10^{c}	0.04
18:1n-11	0.61^{a}	0.01	1.09^{b}	0.04	-	-	0.98^{c}	0.00
18:1n-9	30.60^{ab}	1.58	28.27^{a}	1.07	31.43^{b}	0.88	29.97^{ab}	0.68
18:1n-7	1.29^{a}	0.07	1.98^{b}	0.07	2.11^{b}	0.05	1.68^{c}	0.02
18:2n-6	2.18^{a}	0.08	2.61^{b}	0.10	2.44^{b}	0.02	2.48^{b}	0.03
18:3n-6	0.18^{ac}	0.02	0.14^{b}	0.01	0.15^{b}	0.00	0.20^{c}	0.03
18:3n-3	-	-	0.11	0.00	0.11	0.01	0.10	0.00
18:4n-6	-	-	-	-	-	-	0.12^{a}	0.00
18:4n-3	-	-	-	-	-	-	0.10^{a}	0.00
20:0	0.79^{ab}	0.02	0.71^{a}	0.02	0.86^{b}	0.01	0.75^{a}	0.06
20:1n-11	0.48^{a}	0.02	0.44^{a}	0.02	0.34^{b}	0.02	0.45^{a}	0.02
20:1n-9	2.16^{a}	0.07	2.01^{b}	0.07	2.24^{a}	0.02	1.79^{c}	0.05
20:2n-6	0.20^{a}	0.02	0.14^{b}	0.00	0.15^{b}	0.01	0.13^{b}	0.00
20:4n-6	0.42^{a}	0.01	0.47^{b}	0.01	0.47^{b}	0.00	0.42^{a}	0.01
20:4n-3	-	-	0.11	0.00	0.11	0.00	-	-
20:5n-3	1.79^{a}	0.08	1.71^{a}	0.07	1.76^{a}	0.01	1.18^{b}	0.03
22:0	-	-	-	-	-	-	0.10^{a}	0.00
22:1n-13	-	-	-	-	0.13^{a}	0.00	-	-
22:1n-11	0.57^{ab}	0.03	0.53^{ac}	0.03	0.61^{b}	0.01	0.51^{c}	0.01
22:2n-6	0.19^{ab}	0.01	0.17^{a}	0.01	0.20^{b}	0.01	0.19^{ab}	0.00
22:4n-6	-	-	0.25^{a}	0.02	0.12^{b}	0.01	0.10^{b}	0.00
22:4n-3	0.31^{a}	0.01	0.10^{b}	0.01	0.11^{b}	0.01	0.10^{b}	0.01
22:5n-6	0.12^{a}	0.01	0.23^{b}	0.01	0.16^{c}	0.01	0.24^{b}	0.01
22:5n-3	1.76^{a}	0.11	0.76^{b}	0.05	0.71^{b}	0.03	0.49^{c}	0.02
22:6n-3	25.93^{ab}	0.37	27.91^{a}	1.63	24.41^{ab}	0.20	23.20^{b}	2.18
24:1	-	-	0.13^{a}	0.00	0.10^{b}	0.00	0.14^{a}	0.01
DHA/EPA	14.51^{a}	0.45	16.91^{b}	0.77	13.63^{a}	0.31	20.52^{c}	1.10
EPA/AA	4.27^{a}	0.08	3.67^{b}	0.20	3.80^{b}	0.09	2.79^{c}	0.02
DHA/AA	61.99^{a}	0.76	62.04^{a}	0.51	51.74^{b}	0.06	57.28^{c}	2.72

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. Standard deviation (SD) values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHASCO, DHA-rich single cell oil; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Appendix 5.15. Fatty acid composition (%) of total lipids of rotifers (*Brachionus plicatilis*) enriched for 18 hours with DHASCO emulsion at different concentrations.

Fatty acid	Emulsion concentrations (g/million rotifers)							
	0.1		0.2		0.3		0.4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
12:0	1.26^{a}	0.01	1.28^{a}	0.01	1.70^{b}	0.04	1.00^{c}	0.01
14:0	7.72^{a}	0.15	8.15^{b}	0.12	9.44^{c}	0.20	7.70^{a}	0.13
14:1n-5	0.52^{a}	0.00	0.53^{a}	0.01	0.50^{b}	0.01	0.35^{c}	0.00
15:0	0.20^{a}	0.00	0.18^{b}	0.01	0.18^{b}	0.00	0.16^{c}	0.00
16:0	6.92^{a}	0.11	7.12^{a}	0.12	8.25^{b}	0.15	6.43^{c}	0.13
16:1n-7	9.27	0.22	9.51	0.28	9.22	0.18	8.84	0.34
16:2n-4	0.15^{a}	0.01	0.11^{b}	0.02	0.16^{a}	0.01	0.14^{ab}	0.01
16:3n-4	0.13^{ac}	0.01	0.20^{b}	0.00	0.14^{a}	0.01	0.11^{c}	0.01
17:0	0.14^{a}	0.00	0.16^{ab}	0.02	0.17^{b}	0.00	0.15^{ab}	0.00
17:1	0.29	0.00	0.29	0.00	0.29	0.01	0.28	0.00
18:0	2.08^{ab}	0.01	2.05^{a}	0.02	2.12^{b}	0.01	1.89^{c}	0.02
18:1n-11	1.30^{ab}	0.03	1.39^{a}	0.01	1.20^{bc}	0.10	1.07^{c}	0.01
18:1n-9	29.84^{a}	0.61	31.11^{a}	0.49	30.33^{a}	0.59	27.83^{b}	0.34
18:1n-7	1.91	0.09	2.01	0.06	1.92	0.05	2.03	0.03
18:2n-6	2.96^{a}	0.02	2.96^{a}	0.00	2.53^{b}	0.02	2.47^{c}	0.03
18:3n-6	0.27^{a}	0.04	0.24^{ab}	0.00	0.23^{b}	0.02	0.16^{c}	0.01
18:3n-3	-	-	0.11	0.00	0.11	0.00	0.11	0.01
18:4n-6	-	-	-	-	-	-	0.10^{a}	0.01
18:4n-3	-	-	0.15^{a}	0.01	-	-	-	-
20:0	0.84	0.04	0.84	0.02	0.83	0.04	0.78	0.06
20:1n-11	0.46^{a}	0.00	0.48^{a}	0.01	0.51^{b}	0.00	0.46^{a}	0.02
20:1n-9	2.07^{a}	0.03	2.14^{b}	0.03	2.07^{a}	0.02	2.03^{a}	0.02
20:2n-6	-	-	-	-	-	-	0.14^{a}	0.00
20:4n-6	0.53^{a}	0.04	0.51^{ab}	0.01	0.46^{b}	0.01	0.52^{a}	0.00
20:4n-3	-	-	-	-	-	-	0.11^{a}	0.00
20:5n-3	1.61^{a}	0.02	1.55^{b}	0.03	1.49^{c}	0.00	1.78^{d}	0.01
22:1n-13	-	-	-	-	-	-	0.11^{a}	0.00
22:1n-11	0.58^{a}	0.01	0.59^{a}	0.01	0.57^{a}	0.01	0.52^{b}	0.01
22:2n-6	0.10^{a}	0.00	0.19^{bc}	0.00	0.20^{b}	0.00	0.18^{c}	0.00
22:4n-6	-	-	-	-	-	-	0.12^{a}	0.01
22:5n-6	0.27^{a}	0.03	0.26^{ab}	0.01	0.22^{b}	0.01	0.22^{b}	0.01
22:5n-3	0.50^{a}	0.01	0.50^{a}	0.01	0.52^{a}	0.01	0.64^{b}	0.00
22:6n-3	24.16^{a}	0.66	23.31^{a}	0.44	21.99^{b}	0.41	28.46^{c}	0.22
DHA/EPA	14.96^{a}	0.06	15.07^{a}	0.21	14.78^{a}	0.30	16.02^{b}	0.18
EPA/AA	3.07^{a}	0.20	3.03^{a}	0.14	3.23^{ab}	0.04	3.42^{b}	0.03
DHA/AA	45.99^{a}	3.20	45.61^{a}	1.53	47.78^{a}	1.52	54.71^{b}	0.20

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. Standard deviation (SD) values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHASCO, DHA-rich single cell oil; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Appendix 5.16. Fatty acid composition (%) of total lipids of rotifers (*Brachionus plicatilis*) enriched for 24 hours with DHASCO emulsion at different concentrations.

Fatty acid	Emulsion concentrations (g/million rotifers)							
	0.1		0.2		0.3		0.4	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
12:0	0.82[a]	0.07	1.11[b]	0.09	1.02[b]	0.11	1.12[b]	0.14
14:0	7.18	0.63	7.74	0.50	7.88	0.42	8.22	0.61
14:1n-5	0.44	0.10	0.43	0.12	0.42	0.09	0.40	0.06
15:0	0.20[a]	0.00	0.18[ab]	0.01	0.17[b]	0.00	0.16[b]	0.01
16:0	6.91	0.10	6.61	0.60	7.11	0.55	8.18	0.59
16:1n-7	9.24[a]	0.13	8.51[b]	0.35	9.25[a]	0.11	8.41[b]	0.50
16:2n-4	0.16	0.05	0.12	0.02	0.16	0.03	0.16	0.02
16:3n-4	0.11	0.05	0.15	0.05	0.12	0.06	0.10	0.02
17:0	0.16	0.02	0.16	0.01	0.17	0.01	0.15	0.00
17:1	0.30	0.02	0.29	0.01	0.30	0.01	0.26	0.02
18:0	2.04	0.06	1.96	0.12	2.04	0.10	2.00	0.13
18:1n-11	1.29[ab]	0.06	1.24[a]	0.18	1.14[a]	0.10	1.03[b]	0.05
18:1n-9	30.22	1.01	29.06	1.11	30.88	0.68	28.90	1.30
18:1n-7	1.95	0.17	1.99	0.04	2.02	0.11	1.85	0.20
18:2n-6	2.76	0.23	2.79	0.21	2.48	0.05	2.48	0.03
18:3n-6	0.22	0.06	0.19	0.06	0.19	0.05	0.18	0.04
18:3n-3	0.12	0.02	0.11	0.01	0.11	0.00	0.10	0.01
18:4n-6	-	-	0.10	0.01	-	-	0.11	0.02
18:4n-3	0.12	0.01	0.11	0.05	-	-	0.10	0.02
20:0	0.81	0.04	0.78	0.08	0.85	0.03	0.77	0.05
20:1n-11	0.47	0.01	0.46	0.03	0.42	0.06	0.45	0.02
20:1n-9	2.12	0.07	2.08	0.09	2.16	0.10	1.91	0.14
20:2n-6	0.16	0.02	0.15	0.01	0.15	0.00	0.13	0.01
20:4n-6	0.45	0.01	0.49	0.02	0.47	0.01	0.50	0.03
20:4n-3	0.13	0.01	0.11	0.01	0.11	0.01	0.10	0.01
20:5n-3	1.70	0.11	1.63	0.10	1.69	0.13	1.63	0.18
22:0	-	-	-	-	-	-	0.10	0.01
22:1n-13	-	-	-	-	0.12	0.01	0.10	0.01
22:1n-11	0.57[ab]	0.02	0.56[ab]	0.04	0.59[b]	0.02	0.52[a]	0.01
22:2n-6	0.18	0.02	0.18	0.01	0.20	0.00	0.19	0.01
22:4n-6	-	-	0.12	0.01	0.12	0.01	0.11	0.01
22:4n-3	0.42[a]	0.04	0.10[b]	0.01	0.12[b]	0.01	0.10[b]	0.00
22:5n-6	0.25	0.03	0.24	0.02	0.19	0.05	0.23	0.01
22:5n-3	1.13[a]	0.17	0.63[b]	0.18	0.61[b]	0.12	0.56[b]	0.09
22:6n-3	25.05[ab]	1.05	26.86[a]	1.53	24.41[b]	0.33	26.20[a]	1.17
24:1	-	-	-	-	0.11	0.02	0.13	0.03
DHA/EPA	14.74[a]	0.37	16.51[b]	0.94	14.50[ab]	1.05	16.19[b]	1.10
EPA/AA	3.82[a]	0.18	3.32[b]	0.22	3.63[ab]	0.27	3.28[b]	0.16
DHA/AA	56.28[a]	1.81	54.72[ab]	1.20	52.39[b]	1.32	52.98[b]	1.93

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. Standard deviation (SD) values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHASCO, DHA-rich single cell oil; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Appendix 6.1. Fatty acid composition (%) of total lipids of *Artemia franciscana* nauplii enriched for 6 hours with seal oil emulsion at different concentrations.

Fatty acid	Emulsion concentrations (g/million *Artemia*)							
	1		2		3		4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14:0	1.15[a]	0.01	1.19[a]	0.02	1.30[b]	0.04	1.40[c]	0.01
14:1n-5	0.79[a]	0.00	0.73[b]	0.01	0.77[a]	0.01	0.77[a]	0.01
15:0	0.49[a]	0.01	0.44[b]	0.01	0.44[b]	0.01	0.44[b]	0.01
16:0	9.67	0.34	9.64	0.40	9.54	0.37	9.25	0.17
16:1n-7	6.54[a]	0.37	6.87[ab]	0.31	7.21[b]	0.12	7.52[b]	0.11
16:2n-4	0.43	0.02	0.44	0.00	0.42	0.01	0.42	0.01
16:3n-4	0.70	0.07	0.75	0.01	0.64	0.06	0.66	0.07
16:4n-3	0.43[a]	0.00	0.43[a]	0.00	0.39[b]	0.00	0.41[c]	0.01
17:0	0.82[a]	0.07	0.65[b]	0.01	0.63[b]	0.00	0.68[b]	0.01
17:1	0.51[a]	0.02	0.62[b]	0.02	0.59[b]	0.01	0.63[b]	0.01
18:0	4.48	0.26	4.51	0.38	4.11	0.16	4.07	0.26
18:1n-9	21.10	1.81	21.45	0.63	21.14	0.26	20.62	0.28
18:1n-7	5.22	0.31	5.29	0.20	5.15	0.15	5.24	0.21
18:2n-6	4.54	0.44	4.50	0.13	4.37	0.15	4.19	0.17
18:3n-6	0.42	0.01	0.38	0.04	0.43	0.10	0.46	0.09
18:3n-3	23.80	1.11	22.75	0.98	22.37	0.89	21.73	0.43
18:4n-6	3.77	0.20	3.67	0.17	3.60	0.20	3.63	0.22
20:0	0.77	0.05	0.75	0.04	0.72	0.03	0.78	0.08
20:1n-9	2.44[a]	0.14	2.66[b]	0.09	2.76[bc]	0.08	2.97[c]	0.07
20:4n-6	0.81	0.13	0.80	0.12	0.75	0.15	0.78	0.13
20:4n-3	0.67	0.08	0.67	0.01	0.66	0.01	0.67	0.06
20:5n-3	4.18	0.36	4.28	0.24	4.22	0.16	4.35	0.27
22:0	0.30[a]	0.00	0.29[a]	0.01	0.35[b]	0.01	0.31[a]	0.01
22:5n-3	0.94	0.12	1.04	0.11	1.12	0.10	1.13	0.08
22:6n-3	1.68	0.13	1.82	0.15	1.92	0.11	2.02	0.18
DHA/EPA	0.40	0.01	0.43	0.02	0.45	0.04	0.47	0.03
EPA/AA	5.15	0.32	5.34	0.43	5.59	0.37	5.61	0.22
DHA/AA	2.08[a]	0.18	2.27[ab]	0.20	2.54[ab]	0.16	2.61[b]	0.23

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. Standard deviation (SD) values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Appendix 6.2. Fatty acid composition (%) of total lipids of *Artemia franciscana* nauplii enriched for 12 hours with seal oil emulsion at different concentrations.

Fatty acid	Emulsion concentrations (g/million *Artemia*)							
	1		2		3		4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14:0	1.11^{a}	0.11	1.22^{ab}	0.03	1.37^{b}	0.00	1.68^{c}	0.05
14:1n-5	0.73	0.08	0.73	0.01	0.71	0.01	0.74	0.02
15:0	0.44^{a}	0.00	0.44^{a}	0.01	0.41^{b}	0.00	0.38^{c}	0.01
16:0	9.34^{ab}	0.12	9.41^{a}	0.17	9.24^{ab}	0.22	8.78^{b}	0.34
16:1n-7	6.82^{a}	0.16	7.30^{a}	0.12	7.90^{b}	0.12	8.99^{c}	0.35
16:2n-4	0.41	0.00	0.51	0.08	0.48	0.05	0.49	0.04
16:3n-4	0.66^{a}	0.05	0.75^{a}	0.05	0.71^{a}	0.06	0.42^{b}	0.08
16:4n-3	0.60^{a}	0.04	0.42^{b}	0.02	0.39^{bc}	0.01	0.34^{c}	0.01
17:0	0.61	0.02	0.63	0.01	0.60	0.01	0.60	0.02
17:1	0.88	0.07	0.86	0.05	0.89	0.06	0.87	0.07
18:0	4.46^{a}	0.12	4.62^{a}	0.14	4.04^{b}	0.18	3.68^{b}	0.12
18:1n-9	21.89	0.27	21.83	0.45	21.81	0.60	21.37	0.65
18:1n-7	5.35	0.14	5.42	0.11	5.32	0.27	5.28	0.28
18:2n-6	4.55^{ac}	0.12	4.40^{a}	0.11	5.34^{b}	0.25	4.89^{c}	0.15
18:3n-6	0.63	0.07	0.65	0.01	0.66	0.09	0.71	0.06
18:3n-3	22.14^{a}	0.13	21.28^{b}	0.25	20.22^{c}	0.33	17.97^{d}	0.41
18:4n-6	3.53^{a}	0.10	3.39^{ab}	0.09	$3.23b^{c}$	0.16	3.00^{c}	0.05
20:0	0.80	0.01	0.79	0.01	0.74	0.06	0.79	0.07
20:1n-9	2.58^{a}	0.05	2.94^{b}	0.03	3.28^{c}	0.18	4.23^{d}	0.11
20:2n-6	0.53^{a}	0.01	0.59^{ab}	0.01	0.61^{ab}	0.04	0.70^{b}	0.08
20:4n-6	0.78	0.02	0.77	0.06	0.73	0.05	0.84	0.07
20:4n-3	0.64	0.01	0.65	0.08	0.64	0.07	0.63	0.06
20:5n-3	4.67	0.14	4.78	0.12	4.69	0.15	4.96	0.12
22:0	0.34^{a}	0.01	0.33^{a}	0.01	0.41^{b}	0.01	0.47^{c}	0.01
22:5n-3	1.12^{a}	0.08	1.19^{ab}	0.05	1.32^{b}	0.08	1.58^{c}	0.09
22:6n-3	1.88^{a}	0.12	2.06^{ab}	0.12	2.28^{b}	0.11	2.91^{c}	0.14
DHA/EPA	0.40^{a}	0.00	0.43^{a}	0.01	0.49^{b}	0.02	0.59^{c}	0.01
EPA/AA	5.95^{ac}	0.13	6.22^{abc}	0.13	6.44^{b}	0.16	5.90^{c}	0.22
DHA/AA	2.40^{a}	0.12	2.69^{a}	0.09	3.13^{b}	0.10	3.47^{c}	0.13

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. Standard deviation (SD) values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Appendix 6.3. Fatty acid composition (%) of total lipids of *Artemia franciscana* nauplii enriched for 18 hours with seal oil emulsion at different concentrations.

Fatty acid	Emulsion concentrations (g/million *Artemia*)							
	1		2		3		4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14:0	1.02[a]	0.03	1.15b[c]	0.02	1.10[b]	0.02	1.17[c]	0.00
14:1n-5	1.33[a]	0.04	0.69[b]	0.01	0.69[b]	0.02	0.66[b]	0.00
15:0	0.14[a]	0.00	0.19[b]	0.00	0.19[b]	0.01	0.18[b]	0.00
16:0	11.46[a]	0.33	8.86[b]	0.60	8.88[b]	0.16	8.55[b]	0.23
16:1n-7	4.24[a]	0.12	7.28[b]	0.08	7.16[b]	0.15	7.79[c]	0.12
16:2n-4	0.73[a]	0.02	0.51[b]	0.00	0.47[b]	0.07	0.48[b]	0.00
16:3n-4	1.04[a]	0.03	0.79[b]	0.01	0.79[b]	0.06	0.79[b]	0.00
16:4n-3	0.43[a]	0.01	0.31[b]	0.00	0.29[b]	0.03	0.31[b]	0.00
17:0	0.65[a]	0.01	0.43[bc]	0.00	0.44[b]	0.02	0.41[c]	0.00
17:1	0.36[a]	0.01	0.35[a]	0.01	0.65[b]	0.01	0.59[c]	0.01
18:0	4.80[a]	0.14	4.23[bc]	0.03	4.37[b]	0.07	4.04[c]	0.03
18:1n-9	15.24[a]	0.16	22.39[b]	0.17	22.17[b]	0.51	22.42[b]	0.15
18:1n-7	5.54[a]	0.09	6.05[b]	0.04	6.05[b]	0.24	5.93[b]	0.03
18:2n-6	5.20[a]	0.16	3.98[b]	0.04	4.02[b]	0.09	3.97[b]	0.01
18:3n-6	0.99[a]	0.03	0.21[b]	0.00	0.23[b]	0.00	0.23[b]	0.01
18:3n-3	27.74[a]	0.62	19.56[b]	0.50	20.13[b]	0.36	19.21[b]	0.72
18:4n-6	4.37[a]	0.11	2.93[b]	0.03	2.99[b]	0.05	2.87[b]	0.01
20:0	0.28[a]	0.02	0.59[b]	0.01	0.54[c]	0.01	0.56b[c]	0.00
20:1n-9	3.48	0.02	3.57	0.03	3.40	0.05	3.65	0.04
20:4n-6	1.46[a]	0.11	0.97[b]	0.00	0.93[b]	0.03	0.95[b]	0.01
20:4n-3	0.52[a]	0.04	0.70[b]	0.00	0.66[b]	0.02	0.67[b]	0.00
20:5n-3	5.71[a]	0.13	5.81[a]	0.05	5.21[b]	0.10	5.73[a]	0.04
22:0	0.41	0.03	0.45	0.01	0.45	0.02	0.43	0.01
22:1n-11	-	-	0.34[a]	0.01	0.35[a]	0.01	0.40[b]	0.01
22:5n-3	0.94[a]	0.02	1.48[b]	0.02	1.33[c]	0.01	1.61[d]	0.02
22:6n-3	1.78[a]	0.05	2.49[b]	0.03	2.29[c]	0.03	2.74[d]	0.03
DHA/EPA	0.31[a]	0.02	0.43[b]	0.00	0.44[b]	0.01	0.48[c]	0.00
EPA/AA	3.92[a]	0.19	6.01[b]	0.03	5.58[c]	0.09	6.06[c]	0.02
DHA/AA	1.22[a]	0.12	2.58[b]	0.02	2.45[b]	0.12	2.89[c]	0.00

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. Standard deviation (SD) values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Appendix 6.4. Fatty acid composition (%) of total lipids of *Artemia franciscana* nauplii enriched for 24 hours with seal oil emulsions at different concentrations.

Fatty acid	Emulsion concentrations (g/million *Artemia*)							
	1		2		3		4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14:0	1.08^{ab}	0.03	1.02^{a}	0.03	1.15^{b}	0.05	1.10^{ab}	0.01
14:1n-5	0.70^{a}	0.00	0.69^{ab}	0.01	0.67^{bc}	0.02	0.66^{c}	0.00
15:0	0.28^{a}	0.02	0.35^{a}	0.03	0.34^{c}	0.04	0.29^{a}	0.00
16:0	9.35^{a}	0.24	9.10^{ab}	0.29	8.59^{b}	0.22	8.56^{b}	0.19
16:1n-7	6.98^{a}	0.18	6.49^{b}	0.19	7.55^{c}	0.19	7.37^{ac}	0.15
16:2n-4	0.47	0.05	0.52	0.04	0.47	0.04	0.49	0.00
16:3n-4	0.82	0.08	0.90	0.06	0.83	0.03	0.75	0.00
16:4n-3	-	-	0.15^{a}	0.01	0.29^{b}	0.00	0.29^{b}	0.00
17:0	0.46^{a}	0.00	0.48^{a}	0.01	0.43^{b}	0.00	0.42^{b}	0.00
17:1	0.62^{a}	0.01	0.55^{b}	0.04	0.57^{ab}	0.01	0.57^{ab}	0.01
18:0	4.94^{a}	0.23	4.90^{ab}	0.12	4.87^{ab}	0.36	4.32^{b}	0.15
18:1n-9	23.34	0.20	23.26	0.56	22.61	0.39	22.79	0.25
18:1n-7	6.37^{a}	0.13	6.24^{ab}	0.06	6.06^{b}	0.03	6.09^{b}	0.06
18:2n-6	4.35^{a}	0.07	4.21^{ab}	0.11	4.02^{b}	0.06	4.02^{b}	0.05
18:3n-6	-	-	0.10^{a}	0.01	0.10^{a}	0.01	0.21^{b}	0.03
18:3n-3	21.19^{a}	0.24	21.17^{a}	0.65	19.58^{b}	0.38	19.74^{b}	0.19
18:4n-6	3.00	0.06	2.88	0.09	2.86	0.07	2.88	0.04
20:1n-9	3.43	0.06	3.53	0.11	3.51	0.06	3.60	0.05
20:4n-6	1.02	0.15	0.87	0.01	0.90	0.02	0.90	0.01
20:4n-3	0.65^{a}	0.06	0.76^{b}	0.02	0.71^{ab}	0.03	0.70^{ab}	0.01
20:5n-3	5.20	0.17	5.56	0.20	5.65	0.41	5.85	0.16
22:0	-	-	0.33^{a}	0.04	0.66^{b}	0.03	0.67^{b}	0.03
22:1n-11	-	-	0.25^{a}	0.02	0.16^{b}	0.00	0.41^{c}	0.05
22:2n-6	-	-	0.18^{a}	0.00	-	-	0.19^{a}	0.01
22:5n-3	1.39^{a}	0.03	1.32^{a}	0.04	1.56^{b}	0.03	1.58^{b}	0.03
22:6n-3	2.27^{a}	0.07	2.34^{a}	0.13	2.65^{b}	0.03	2.91^{c}	0.03
DHA/EPA	0.44^{ab}	0.03	0.42^{a}	0.02	0.47^{ab}	0.04	0.50^{b}	0.01
EPA/AA	5.15^{a}	0.90	6.41^{ab}	0.07	6.29^{ab}	0.30	6.50^{b}	0.27
DHA/AA	2.24^{a}	0.25	2.70^{b}	0.18	2.95^{bc}	0.11	3.23^{c}	0.08

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. Standard deviation (SD) values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Appendix 6.5. Fatty acid composition (%) of total lipids of *Artemia franciscana* nauplii enriched for 6 hours with menhaden oil emulsion at different concentrations.

Fatty acid	Emulsion concentrations (g/million *Artemia*)							
	1		2		3		4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
12:0	0.14^{a}	0.00	-	-	-	-	-	-
14:0	0.99	0.02	0.94	0.02	0.98	0.02	0.99	0.02
14:1n-5	1.53	0.02	1.56	0.03	1.55	0.03	1.52	0.04
15:0	0.67^{a}	0.01	0.67^{a}	0.01	0.68^{a}	0.02	0.78^{b}	0.02
16:0	12.20	0.19	12.18	0.23	12.39	0.28	12.19	0.28
16:1n-7	4.59	0.07	4.67	0.09	4.73	0.11	4.67	0.11
16:2n-4	0.82	0.01	0.82	0.02	0.83	0.02	0.81	0.02
16:3n-4	1.13	0.02	1.14	0.02	1.14	0.03	1.12	0.03
16:4n-3	0.52^{ab}	0.01	0.52^{ab}	0.01	0.54^{a}	0.01	0.51^{b}	0.01
17:0	0.75	0.01	0.74	0.01	0.74	0.02	0.74	0.02
17:1	0.49^{a}	0.01	0.42^{b}	0.01	0.42^{b}	0.01	0.45^{c}	0.01
18:0	5.72	0.09	5.60	0.10	5.66	0.13	5.60	0.13
18:1n-9	15.74	0.25	15.71	0.29	15.78	0.35	15.80	0.37
18:1n-7	5.86	0.09	5.86	0.11	5.80	0.13	5.81	0.13
18:2n-6	5.92	0.09	6.03	0.11	5.96	0.13	5.93	0.14
18:3n-6	1.02	0.02	1.05	0.02	1.02	0.02	1.01	0.02
18:3n-3	20.29	0.39	21.14	0.36	21.46	0.48	21.75	0.42
18:4n-6	4.37	0.08	4.51	0.08	4.51	0.10	4.43	0.07
20:1n-9	0.49^{ab}	0.01	0.49^{ab}	0.01	0.48^{a}	0.01	0.51^{b}	0.01
20:4n-6	1.33^{ab}	0.03	1.36^{a}	0.02	1.27^{b}	0.03	1.31^{ab}	0.01
20:4n-3	0.48	0.01	0.48	0.01	0.49	0.01	0.49	0.01
20:5n-3	8.42^{a}	0.14	8.62^{a}	0.16	7.63^{b}	0.17	7.67^{b}	0.21
22:1n-11	0.29^{a}	0.01	0.47^{b}	0.01	0.31^{a}	0.01	0.40^{c}	0.01
22:6n-3	2.57^{a}	0.07	2.62^{ab}	0.06	2.55^{a}	0.08	2.79^{b}	0.10
DHA/EPA	0.31^{a}	0.02	0.30^{a}	0.01	0.33^{a}	0.01	0.36^{b}	0.01
EPA/AA	6.34^{ab}	0.25	6.32^{a}	0.05	6.01^{b}	0.03	5.83^{c}	0.01
DHA/AA	1.94^{ab}	0.02	1.92^{a}	0.02	2.01^{b}	0.01	2.12^{c}	0.05

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. Standard deviation (SD) values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Appendix 6.6. Fatty acid composition (%) of total lipids of *Artemia franciscana* nauplii enriched for 12 hours with menhaden oil emulsion at different concentrations.

Fatty acid	Emulsion concentrations (g/million *Artemia*)							
	1		2		3		4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
12:0	0.11^{a}	0.00	0.11^{a}	0.00	1.10^{b}	0.02	0.10^{a}	0.00
14:0	1.53^{a}	0.03	1.70^{b}	0.03	1.71^{b}	0.03	2.00^{c}	0.04
14:1n-5	0.60^{a}	0.01	0.57^{b}	0.01	0.57^{b}	0.01	0.58^{ab}	0.01
15:0	0.36^{a}	0.01	0.28^{b}	0.01	0.27^{b}	0.00	0.28^{b}	0.01
16:0	10.79^{ab}	0.22	11.23^{a}	0.17	10.69^{b}	0.18	11.02^{ab}	0.24
16:1n-7	5.20^{a}	0.10	5.44^{a}	0.11	5.74^{b}	0.10	5.97^{b}	0.13
16:2n-4	0.53^{a}	0.01	0.51^{ab}	0.01	0.50^{b}	0.01	0.50^{b}	0.01
16:3n-4	0.82^{a}	0.02	0.77^{b}	0.02	0.76^{b}	0.01	0.76^{b}	0.02
16:4n-3	0.51^{a}	0.01	0.55^{b}	0.01	0.60^{c}	0.01	0.65^{d}	0.01
17:0	0.52^{a}	0.01	0.50^{ab}	0.01	0.49^{b}	0.01	0.49^{b}	0.01
17:1	0.76^{a}	0.02	0.78^{a}	0.02	0.80^{a}	0.01	0.87^{b}	0.02
18:0	5.22^{a}	0.10	5.05^{ab}	0.10	4.93^{b}	0.09	4.91^{b}	0.11
18:1n-9	17.71	0.35	17.38	0.35	17.49	0.30	17.37	0.38
18:1n-7	5.65	0.11	5.50	0.11	5.55	0.10	5.57	0.12
18:2n-6	3.72	0.07	3.66	0.07	3.61	0.06	3.58	0.08
18:3n-6	0.28^{a}	0.01	0.26^{a}	0.01	0.32^{b}	0.01	0.28^{a}	0.01
18:3n-3	19.61^{a}	0.39	18.56^{b}	0.37	18.13^{b}	0.31	18.25^{b}	0.25
18:4n-6	3.35	0.07	3.46	0.09	3.44	0.06	3.47	0.09
20:0	0.26	0.01	0.26	0.01	0.26	0.00	0.25	0.01
20:1n-9	1.04	0.02	1.06	0.03	1.06	0.02	1.03	0.03
20:4n-6	1.06^{a}	0.02	1.08^{a}	0.01	1.10^{ab}	0.02	1.14^{b}	0.02
20:4n-3	1.14^{a}	0.02	1.23^{b}	0.03	1.20^{ab}	0.02	1.23^{b}	0.03
20:5n-3	8.45^{a}	0.11	9.06^{ab}	0.10	9.25^{b}	0.29	9.03^{ab}	0.36
22:0	0.82^{a}	0.02	0.79^{ab}	0.02	0.77^{b}	0.01	0.76^{b}	0.02
22:1n-11	0.52^{a}	0.01	0.48^{b}	0.01	0.47^{b}	0.01	0.47^{b}	0.01
22:2n-6	0.22^{a}	0.00	0.25^{b}	0.01	0.27^{c}	0.00	0.25^{b}	0.01
22:4n-6	0.32^{a}	0.01	-	-	-	-	-	-
22:4n-3	0.10^{a}	0.00	-	-	-	-	-	-
22:5n-3	0.91^{a}	0.02	1.05^{bc}	0.03	1.10^{b}	0.02	1.03^{c}	0.03
22:6n-3	3.73^{a}	0.16	4.65^{b}	0.15	4.85^{b}	0.15	4.55^{b}	0.15
DHA/EPA	0.44^{a}	0.01	0.51^{b}	0.02	0.52^{b}	0.01	0.50^{b}	0.00
EPA/AA	7.99^{a}	0.06	8.36^{b}	0.19	8.41^{b}	0.06	7.95^{a}	0.18
DHA/AA	3.53^{a}	0.08	4.29^{b}	0.09	4.40^{b}	0.06	4.00^{c}	0.06

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. Standard deviation (SD) values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Appendix 6.7. Fatty acid composition (%) of total lipids of *Artemia franciscana* nauplii enriched for 18 hours with menhaden oil emulsion at different concentrations.

Fatty acid	Emulsion concentrations (g/million *Artemia*)							
	1		2		3		4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14:0	1.35^{a}	0.01	1.54^{b}	0.01	1.79^{c}	0.03	1.73^{c}	0.03
14:1n-5	0.67	0.00	0.64	0.01	0.65	0.02	0.64	0.01
15:0	0.23^{a}	0.00	0.25^{ab}	0.00	0.26^{b}	0.02	0.25^{ab}	0.00
16:0	11.00	0.60	10.75	0.57	11.53	0.29	11.19	0.44
16:1n-7	4.20^{a}	0.06	5.44^{b}	0.04	5.80^{c}	0.13	5.38^{b}	0.03
16:2n-4	0.57^{a}	0.00	0.55^{bc}	0.00	0.56^{ab}	0.01	0.54^{c}	0.00
16:3n-4	0.92	0.00	0.85	0.01	0.86	0.02	0.99	0.21
16:4n-3	0.53^{a}	0.00	0.63^{b}	0.00	0.63^{b}	0.05	0.63^{b}	0.00
17:0	0.56^{a}	0.00	0.53^{b}	0.00	0.54^{b}	0.01	0.53^{b}	0.00
17:1	0.77^{a}	0.01	0.91^{b}	0.00	0.91^{b}	0.02	0.92^{b}	0.01
18:0	5.47^{a}	0.12	4.97^{b}	0.11	5.21^{ab}	0.12	5.27^{ab}	0.13
18:1n-9	18.84	0.90	18.02	0.73	18.21	0.53	18.28	0.60
18:1n-7	5.96	0.12	5.78	0.11	5.70	0.17	5.91	0.12
18:2n-6	4.18	0.11	4.02	0.10	4.00	0.11	4.07	0.09
18:3n-6	0.25^{a}	0.00	0.27^{a}	0.01	0.32^{b}	0.02	0.28^{a}	0.02
18:3n-3	22.46^{a}	0.45	20.32^{b}	0.61	19.73^{b}	0.52	20.12^{b}	0.44
18:4n-6	3.52	0.01	3.44	0.01	3.46	0.09	3.43	0.00
20:0	0.52^{a}	0.01	0.37^{b}	0.00	0.69^{c}	0.04	0.41^{b}	0.01
20:1n-9	1.01^{a}	0.01	0.97^{b}	0.00	1.00^{ab}	0.02	1.01^{a}	0.01
20:4n-6	1.18	0.02	1.13	0.04	1.11	0.05	1.15	0.00
20:4n-3	1.02^{a}	0.00	1.11^{b}	0.00	1.17^{c}	0.00	1.12^{b}	0.01
20:5n-3	6.79^{a}	0.13	7.78^{b}	0.13	7.55^{b}	0.19	6.96^{a}	0.17
22:0	0.58^{a}	0.01	0.52^{b}	0.01	0.73^{c}	0.03	0.53^{b}	0.00
22:1n-11	0.28^{a}	0.01	0.31^{b}	0.00	0.46^{c}	0.01	0.40^{d}	0.01
22:5n-3	0.63^{a}	0.02	0.86^{b}	0.01	0.88^{b}	0.04	0.82^{b}	0.06
22:6n-3	2.49^{a}	0.03	3.70^{b}	0.03	3.68^{b}	0.11	3.39^{c}	0.03
DHA/EPA	0.37^{a}	0.00	0.48^{b}	0.00	0.49^{b}	0.00	0.49^{b}	0.01
EPA/AA	5.77^{a}	0.06	6.86^{b}	0.27	6.84^{b}	0.45	6.05^{a}	0.08
DHA/AA	2.12^{a}	0.00	3.26^{bc}	0.14	3.33^{b}	0.24	2.95^{c}	0.04

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. Standard deviation (SD) values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Appendix 6.8. Fatty acid composition (%) of total lipids of *Artemia franciscana* nauplii enriched for 24 hours with menhaden oil emulsion at different concentrations.

Fatty acid	Emulsion concentrations (g/million *Artemia*)							
	1		2		3		4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14:0	1.23^{a}	0.02	1.39^{ab}	0.12	1.54^{bc}	0.04	1.72^{cb}	0.10
14:1n-5	0.64^{a}	0.01	0.59^{b}	0.02	0.60^{b}	0.00	0.59^{b}	0.01
15:0	0.31	0.13	0.32	0.11	0.27	0.01	0.31	0.05
16:0	10.56^{a}	0.18	11.11^{ab}	0.15	11.39^{b}	0.35	11.60^{b}	0.18
16:1n-7	4.53^{a}	0.25	5.71^{b}	0.12	6.16^{c}	0.10	6.37^{c}	0.09
16:2n-4	0.53	0.02	0.52	0.02	0.48	0.03	0.50	0.00
16:3n-4	0.88	0.03	0.82	0.03	0.90	0.13	0.80	0.04
16:4n-3	0.32^{a}	0.01	0.56^{b}	0.02	0.77^{c}	0.01	0.68^{d}	0.01
17:0	0.53^{a}	0.01	0.51^{ac}	0.00	0.48^{bd}	0.02	0.50^{cd}	0.00
17:1	0.72^{a}	0.02	0.82^{b}	0.01	0.92^{c}	0.04	0.90^{c}	0.01
18:0	5.44^{a}	0.05	5.13^{b}	0.06	$4.93b^{c}$	0.08	4.70^{c}	0.14
18:1n-9	18.46	0.15	18.22	0.13	17.94	0.17	17.96	0.33
18:1n-7	5.77^{a}	0.02	5.60^{ab}	0.07	5.40^{b}	0.06	5.40^{b}	0.18
18:2n-6	4.12^{a}	0.03	4.02^{ab}	0.03	4.04^{ab}	0.03	3.98^{b}	0.08
18:3n-6	0.25^{a}	0.00	0.22^{a}	0.02	0.23^{a}	0.02	0.39^{b}	0.03
18:3n-3	22.54^{a}	0.30	19.85^{b}	0.11	19.11^{c}	0.22	18.99^{c}	0.33
18:4n-6	3.60^{a}	0.05	3.50^{a}	0.07	3.29^{b}	0.07	3.56^{a}	0.07
20:0	0.28^{a}	0.02	0.32^{ab}	0.01	0.33^{b}	0.02	0.31^{ab}	0.01
20:1n-9	1.06	0.02	1.10	0.04	1.08	0.03	1.06	0.03
20:4n-6	1.06	0.04	1.12	0.00	1.13	0.11	1.07	0.04
20:4n-3	1.06^{a}	0.01	1.17^{b}	0.06	1.30^{c}	0.01	1.31^{c}	0.04
20:5n-3	7.22^{a}	0.17	9.14^{b}	0.11	9.01^{bc}	0.20	8.67^{c}	0.18
22:0	0.86^{a}	0.01	0.75^{b}	0.04	0.70^{b}	0.06	0.77^{b}	0.02
22:1n-11	0.50	0.01	0.54	0.04	0.56	0.01	0.51	0.04
22:2n-6	0.20	0.02	0.20	0.00	0.20	0.01	0.18	0.01
22:5n-6	0.37^{a}	0.06	0.27^{ab}	0.04	0.18^{bc}	0.01	0.16^{c}	0.03
22:5n-3	0.72^{a}	0.00	1.16^{b}	0.03	1.12^{b}	0.06	1.03^{c}	0.00
22:6n-3	2.60^{a}	0.09	4.29^{b}	0.12	4.76^{c}	0.13	4.54^{bc}	0.11
DHA/EPA	0.36^{a}	0.00	0.47^{b}	0.01	0.53^{c}	0.03	0.52^{c}	0.00
EPA/AA	6.79^{a}	0.18	8.16^{b}	0.12	8.02^{b}	0.58	8.12^{b}	0.11
DHA/AA	2.45^{a}	0.06	3.83^{b}	0.12	4.24^{b}	0.51	4.25^{b}	0.05

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. Standard deviation (SD) values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Appendix 6.9. Fatty acid composition (%) of total lipids of *Artemia franciscana* nauplii enriched for 6 hours with Algamac-3010 emulsion at different concentrations.

Fatty acid	Emulsion concentrations (g/million *Artemia*)							
	1		2		3		4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
12:0	0.10[a]	0.00	0.10[a]	0.00	0.12[b]	0.00	0.11[ab]	0.00
14:0	1.91	0.10	2.00	0.03	1.94	0.02	2.02	0.05
14:1n-5	0.91	0.00	0.92	0.01	0.92	0.01	0.94	0.02
15:0	0.28[a]	0.02	0.38[b]	0.05	0.38[b]	0.06	0.38[b]	0.06
16:0	12.32	0.31	12.29	0.18	12.20	0.14	12.45	0.13
16:1n-7	2.81	0.01	2.85	0.04	2.84	0.03	2.88	0.07
16:2n-4	0.54	0.00	0.56	0.01	0.55	0.01	0.55	0.01
16:3n-4	0.98	0.00	1.00	0.02	1.00	0.01	1.02	0.03
16:4n-3	0.43	0.00	0.42	0.01	0.42	0.00	0.43	0.01
17:0	0.61[a]	0.00	0.62[a]	0.01	0.72[b]	0.01	0.74[b]	0.02
17:1	0.76[a]	0.00	0.79[a]	0.01	0.85[b]	0.01	0.86[b]	0.02
18:0	4.33	0.07	4.32	0.06	4.25	0.05	4.46	0.11
18:1n-9	17.17[a]	0.61	17.65[ab]	0.38	18.16[ab]	0.20	18.38[b]	0.46
18:1n-7	5.21	0.16	5.23	0.08	5.23	0.06	5.24	0.13
18:2n-6	4.96[a]	0.18	5.28[b]	0.08	5.31[b]	0.06	5.31[b]	0.13
18:3n-6	0.32	0.07	0.28	0.00	0.29	0.00	0.28	0.01
18:3n-3	23.89	1.00	24.77	0.37	24.71	0.28	25.04	0.63
18:4n-6	3.81	0.02	3.82	0.06	3.80	0.04	3.82	0.10
20:1n-9	0.45	0.00	0.46	0.01	0.46	0.01	0.47	0.01
20:4n-6	1.37	0.08	1.37	0.02	1.32	0.03	1.30	0.02
20:4n-3	0.64	0.05	0.61	0.01	0.58	0.01	0.62	0.02
20:5n-3	3.25	0.10	3.38	0.14	3.20	0.04	3.17	0.01
22:0	0.40[a]	0.02	0.52[b]	0.05	0.50[b]	0.05	0.51[b]	0.04
22:1n-11	0.50	0.16	0.52	0.17	0.50	0.14	0.52	0.17
22:5n-3	1.84[a]	0.01	1.81[b]	0.03	1.73[b]	0.02	1.79[ab]	0.04
22:6n-3	4.92	0.20	5.07	0.26	5.07	0.27	4.90	0.19
DHA/EPA	1.51	0.01	1.50	0.01	1.58	0.10	1.54	0.07
EPA/AA	2.37	0.22	2.47	0.07	2.43	0.09	2.43	0.05
DHA/AA	3.59	0.37	3.71	0.13	3.83	0.11	3.76	0.09

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. Standard deviation (SD) values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Appendix 6.10. Fatty acid composition (%) of total lipids of *Artemia franciscana* nauplii enriched for 12 hours with Algamac-3010 emulsion at different concentrations.

Fatty acid	Emulsion concentrations (g/million *Artemia*)							
	1		2		3		4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14:0	1.29^{a}	0.02	1.29^{a}	0.03	1.96^{b}	0.04	1.59^{c}	0.04
14:1n-5	1.47^{a}	0.02	1.44^{ab}	0.03	1.51^{a}	0.03	1.38^{b}	0.03
15:0	0.78^{a}	0.01	0.64^{b}	0.01	0.83^{c}	0.02	0.73^{d}	0.02
16:0	12.50	0.18	12.69	0.29	12.95	0.27	12.59	0.29
16:1n-7	4.40	0.11	4.45	0.10	4.53	0.09	4.36	0.10
16:2n-4	0.78^{a}	0.01	0.89^{b}	0.02	0.82^{a}	0.02	0.81^{a}	0.02
16:3n-4	1.13^{a}	0.02	1.10^{ab}	0.03	1.09^{ab}	0.02	1.05^{b}	0.02
16:4n-3	0.53^{a}	0.01	0.50^{b}	0.01	0.48^{bc}	0.01	0.47^{c}	0.01
17:0	0.73^{a}	0.01	0.72^{ab}	0.02	0.71^{ab}	0.01	0.68^{b}	0.02
17:1	1.02^{a}	0.01	1.08^{b}	0.03	0.95^{c}	0.02	0.88^{d}	0.02
18:0	6.07^{a}	0.09	5.87^{a}	0.14	5.79^{ab}	0.12	5.49^{b}	0.13
18:1n-9	14.83^{a}	0.21	14.49^{ab}	0.34	13.99^{b}	0.30	14.07^{ab}	0.33
18:1n-7	6.10^{a}	0.09	5.96^{ab}	0.14	5.74^{b}	0.12	5.73^{b}	0.13
18:2n-6	4.71^{ab}	0.12	4.70^{ab}	0.11	4.53^{a}	0.11	4.95^{b}	0.11
18:3n-6	1.09^{a}	0.02	1.04^{ab}	0.02	1.02^{b}	0.02	1.10^{a}	0.03
18:3n-3	22.95^{a}	0.49	21.98^{ab}	0.51	21.35^{b}	0.46	22.29^{ab}	0.52
18:4n-6	3.68^{a}	0.08	3.48^{ab}	0.08	3.41^{b}	0.07	3.58^{ab}	0.08
20:1n-9	0.48^{a}	0.01	0.46^{a}	0.01	0.46^{a}	0.01	0.54^{b}	0.01
20:4n-6	1.53	0.05	1.56	0.04	1.53	0.04	1.59	0.04
20:4n-3	0.52^{a}	0.01	0.51^{a}	0.01	0.47^{b}	0.01	0.48^{b}	0.01
20:5n-3	4.57^{a}	0.11	4.44^{a}	0.11	4.52^{a}	0.08	4.92^{b}	0.09
22:0	0.47^{a}	0.01	0.47^{a}	0.01	0.41^{b}	0.01	0.47^{a}	0.01
22:5n-3	0.66^{a}	0.02	0.69^{a}	0.02	0.61^{b}	0.01	0.85^{c}	0.01
22:6n-3	5.88^{a}	0.12	6.19^{ab}	0.21	6.59^{b}	0.23	6.67^{b}	0.22
DHA/EPA	1.29^{a}	0.06	1.40^{ab}	0.08	1.46^{b}	0.02	1.35^{ab}	0.02
EPA/AA	2.99	0.17	2.84	0.14	2.96	0.14	3.10	0.02
DHA/AA	3.85^{a}	0.04	3.96^{ab}	0.04	4.32^{b}	0.27	4.20^{a}	0.04

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. Standard deviation (SD) values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Appendix 6.11. Fatty acid composition (%) of total lipids of *Artemia franciscana* nauplii enriched for 18 hours with Algamac-3010 emulsion at different concentrations.

Fatty acid	Emulsion concentrations (g/million *Artemia*)							
	1		2		3		4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14:0	1.00	0.01	0.96	0.05	0.99	0.04	1.01	0.07
14:1n-5	0.71^{a}	0.00	1.31^{b}	0.07	1.28^{b}	0.04	1.28^{b}	0.08
15:0	0.19^{a}	0.00	0.22^{a}	0.02	0.15^{b}	0.02	0.15^{b}	0.01
16:0	9.14^{a}	0.32	10.03^{ab}	0.48	10.02^{ab}	0.29	10.58^{b}	0.30
16:1n-7	6.28^{a}	0.00	4.15^{b}	0.20	4.13^{b}	0.13	4.23^{b}	0.23
16:2n-4	0.53^{a}	0.01	0.72^{b}	0.03	0.71^{b}	0.02	0.71^{b}	0.04
16:3n-4	0.86^{a}	0.00	1.04^{b}	0.04	1.03^{b}	0.03	1.01^{b}	0.05
16:4n-3	0.28^{a}	0.00	0.42^{b}	0.02	0.41^{b}	0.02	0.42^{b}	0.03
17:0	0.47^{a}	0.00	0.64^{b}	0.03	0.64^{b}	0.02	0.63^{b}	0.03
17:1	0.36	0.01	0.36	0.02	0.35	0.01	0.40	0.03
18:0	4.67	0.02	4.83	0.18	4.76	0.15	4.73	0.21
18:1n-9	19.36^{a}	0.64	14.69^{b}	0.56	14.56^{b}	0.42	14.57^{b}	0.61
18:1n-7	6.15^{a}	0.01	5.48^{b}	0.22	5.42^{b}	0.15	5.45^{b}	0.25
18:2n-6	4.13^{a}	0.01	5.13^{b}	0.21	5.12^{b}	0.16	5.24^{b}	0.02
18:3n-6	0.23^{a}	0.00	0.99^{b}	0.04	0.99^{b}	0.03	1.01^{b}	0.01
18:3n-3	21.61^{a}	0.89	25.63^{b}	1.93	25.01^{b}	0.70	25.41^{b}	1.24
18:4n-6	3.16^{a}	0.01	4.32^{b}	0.17	4.29^{b}	0.14	4.15^{b}	0.21
20:0	0.25^{a}	0.06	0.36^{b}	0.02	0.30^{ab}	0.01	0.38^{b}	0.02
20:1n-9	3.01^{a}	0.02	0.46^{b}	0.03	0.46^{b}	0.02	0.69^{c}	0.01
20:4n-6	0.96^{a}	0.03	1.41^{b}	0.06	1.42^{b}	0.08	1.47^{b}	0.04
20:4n-3	0.75^{a}	0.04	0.50^{b}	0.02	0.49^{b}	0.02	0.50^{b}	0.01
20:5n-3	5.31	0.02	5.22	0.21	5.32	0.06	5.48	0.09
22:0	0.50^{a}	0.00	0.42^{b}	0.00	0.41^{b}	0.04	0.43^{b}	0.01
22:4n-3	0.40^{a}	0.03	-	-	-	-	-	-
22:5n-3	1.13^{a}	0.09	-	-	-	-	-	-
22:6n-3	4.61^{a}	0.40	5.55^{b}	0.37	5.91^{bc}	0.05	6.48^{c}	0.29
DHA/EPA	0.87^{a}	0.08	1.06^{b}	0.03	1.11^{b}	0.02	1.18^{b}	0.07
EPA/AA	5.53^{a}	0.18	3.71^{b}	0.30	3.77^{b}	0.37	3.73^{b}	0.04
DHA/AA	4.79	0.28	3.94	0.42	4.19	0.49	4.42	0.32

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. Standard deviation (SD) values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Appendix 6.12. Fatty acid composition (%) of total lipids of *Artemia franciscana* nauplii enriched for 24 hours with Algamac-3010 emulsion at different concentrations.

Fatty acid	Emulsion concentrations (g/million *Artemia*)							
	1		2		3		4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14:0	1.77^{a}	0.03	2.13^{b}	0.01	2.25^{c}	0.03	2.34^{d}	0.05
14:1n-5	1.25^{a}	0.00	1.27^{a}	0.07	1.15^{a}	0.05	1.16^{b}	0.01
15:0	0.58^{a}	0.01	0.57^{a}	0.04	0.58^{a}	0.06	0.37^{b}	0.03
16:0	13.22	0.22	13.80	0.43	13.27	0.12	13.79	0.23
16:1n-7	4.13	0.08	4.14	0.06	3.98	0.08	3.99	0.11
16:2n-4	0.68^{a}	0.01	0.74^{b}	0.01	0.63^{c}	0.00	0.71^{ab}	0.02
16:3n-4	1.00	0.05	0.97	0.05	0.92	0.04	0.94	0.06
16:4n-3	0.39^{a}	0.01	-	-	0.37^{ab}	0.01	0.35^{b}	0.01
17:0	0.45	0.08	0.59	0.03	0.46	0.01	0.48	0.01
17:1	0.48^{a}	0.07	0.36^{b}	0.02	0.21^{c}	0.04	0.25^{c}	0.05
18:0	5.74	0.25	5.48	0.28	5.28	0.14	5.38	0.12
18:1n-9	14.19^{a}	0.19	13.72^{b}	0.21	12.93^{c}	0.14	13.38^{bc}	0.14
18:1n-7	5.92^{a}	0.05	5.76^{b}	0.01	5.46^{c}	0.04	5.62^{d}	0.05
18:2n-6	5.07^{a}	0.04	4.85^{b}	0.00	4.66^{c}	0.03	4.71^{c}	0.04
18:3n-6	0.92	0.01	0.90	0.01	0.89	0.03	0.89	0.04
18:3n-3	23.32^{ab}	0.26	23.45^{a}	0.44	22.56^{bc}	0.20	22.49^{c}	0.29
18:4n-6	3.91^{a}	0.05	3.69^{b}	0.03	3.67^{b}	0.03	3.70^{b}	0.05
20:1n-9	0.43^{a}	0.02	0.59^{b}	0.03	0.75^{c}	0.05	0.45^{a}	0.04
20:4n-6	2.06^{a}	0.04	2.28^{b}	0.06	2.24^{b}	0.08	2.24^{b}	0.02
20:4n-3	0.44^{a}	0.03	0.58^{b}	0.04	0.41^{a}	0.01	0.40^{a}	0.01
20:5n-3	3.52^{a}	0.01	3.85^{b}	0.04	3.77^{b}	0.06	3.66^{c}	0.03
22:0	0.53^{a}	0.02	0.47^{ab}	0.02	0.55^{a}	0.01	0.42^{b}	0.02
22:5n-3	1.69^{a}	0.05	2.51^{b}	0.10	2.56^{b}	0.06	2.49^{b}	0.03
22:6n-3	4.54^{a}	0.03	5.19^{b}	0.04	6.11^{c}	0.03	5.97^{d}	0.08
DHA/EPA	1.29^{a}	0.01	1.35^{b}	0.01	1.62^{c}	0.03	1.63^{c}	0.01
EPA/AA	1.71^{a}	0.03	1.69^{ab}	0.04	1.68^{ab}	0.03	1.63^{b}	0.00
DHA/AA	2.21^{a}	0.03	2.27^{a}	0.05	2.73^{b}	0.01	2.66^{b}	0.01

Analyses were carried put in triplicates. – Mean values <0.1 or not detected. Standard deviation (SD) values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Appendix 6.13. Fatty acid composition (%) of total lipids of *Artemia franciscana* nauplii enriched for 6 hours with DHASCO emulsions at different concentrations.

Fatty acid	Emulsion concentrations (g/million *Artemia*)							
	0.5		1.0		1.5		2.0	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
12:0	0.43^{a}	0.01	0.50^{b}	0.01	0.56^{c}	0.03	0.52^{bc}	0.01
14:0	2.23^{a}	0.00	2.40^{b}	0.02	2.62^{c}	0.04	2.52^{d}	0.02
14:1n-5	0.89	0.00	0.87	0.01	0.88	0.01	0.88	0.01
15:0	0.22^{a}	0.01	0.20^{b}	0.00	0.32^{c}	0.00	0.24^{d}	0.01
16:0	10.31^{a}	0.02	10.29^{a}	0.09	10.49^{b}	0.07	10.41^{ab}	0.04
16:1n-7	3.31^{a}	0.01	3.25^{b}	0.02	3.75^{c}	0.02	3.39^{d}	0.02
16:2n-4	0.60	0.08	0.59	0.08	0.65	0.00	0.54	0.00
16:3n-4	1.35^{a}	0.01	1.33^{ab}	0.01	1.31^{b}	0.00	1.33^{ab}	0.01
16:4n-3	0.09^{a}	0.02	0.13^{a}	0.04	-	-	-	-
17:0	0.67^{a}	0.02	0.65^{a}	0.01	0.80^{b}	0.00	0.75^{c}	0.00
17:1	0.79^{ab}	0.01	0.76^{a}	0.03	0.83^{b}	0.03	0.79^{ab}	0.00
18:0	4.00^{a}	0.01	3.93^{b}	0.02	3.88^{c}	0.01	3.88^{c}	0.01
18:1n-9	20.85	0.77	20.96	0.37	20.62	0.44	20.75	0.64
18:1n-7	4.72	0.05	4.68	0.22	4.58	0.10	4.52	0.02
18:2n-6	5.10	0.12	5.11	0.02	4.97	0.02	5.00	0.01
18:3n-6	0.24	0.01	0.24	0.00	0.23	0.00	0.23	0.00
18:3n-3	23.79	0.90	23.51	0.75	22.66	0.90	23.25	0.55
18:4n-6	3.53^{a}	0.01	3.48^{b}	0.02	3.41^{c}	0.01	3.46^{b}	0.01
18:4n-3	0.14	0.00	0.14	0.00	0.15	0.00	0.14	0.00
20:0	0.11^{a}	0.00	0.13^{b}	0.00	0.11^{a}	0.00	0.15^{c}	0.00
20:1n-9	0.52	0.00	0.52	0.00	0.52	0.00	0.54	0.00
20:2n-6	0.17	0.00	0.17	0.00	0.16	0.01	0.17	0.00
20:4n-6	0.85^{a}	0.00	0.84^{a}	0.00	0.80^{b}	0.01	0.82^{c}	0.00
20:4n-3	0.64	0.00	0.68	0.00	0.62	0.05	0.65	0.03
20:5n-3	2.84^{a}	0.01	2.72^{b}	0.00	2.65^{c}	0.02	2.72^{b}	0.00
22:0	0.42	0.00	0.41	0.00	0.42	0.00	0.42	0.00
22:4n-3	0.23^{a}	0.00	0.20^{b}	0.01	0.20^{b}	0.00	0.22^{a}	0.00
22:5n-3	0.12	0.03	0.12	0.03	0.14	0.06	0.16	0.03
22:6n-3	6.26^{a}	0.25	6.84^{bc}	0.14	6.86^{b}	0.15	6.39^{ac}	0.14
DHA/EPA	2.20^{a}	0.10	2.51^{bc}	0.02	2.59^{b}	0.06	2.35^{ac}	0.05
EPA/AA	3.36	0.03	3.24	0.01	3.32	0.07	3.32	0.04
DHA/AA	7.40^{a}	0.32	8.14^{bc}	0.03	8.60^{b}	0.16	7.81^{ac}	0.19

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. Standard deviation (SD) values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHASCO, DHA-rich single cell oil; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Appendix 6.14. Fatty acid composition (%) of total lipids of *Artemia franciscana* nauplii enriched for 12 hours with DHASCO emulsions at various concentrations.

Fatty acid	Emulsion concentrations (g/million *Artemia*)							
	0.5		1.0		1.5		2.0	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
12:0	0.28^{a}	0.00	0.41^{b}	0.01	0.41^{b}	0.00	0.51^{c}	0.01
14:0	1.49^{a}	0.00	1.94^{b}	0.02	1.93^{b}	0.04	2.24^{c}	0.01
14:1n-5	0.88^{a}	0.00	0.76^{b}	0.01	0.79^{c}	0.00	0.75^{b}	0.01
15:0	0.22^{a}	0.01	0.18^{b}	0.01	0.19^{b}	0.00	0.18^{b}	0.00
16:0	10.26	0.62	9.57	0.38	9.77	0.41	9.60	0.36
16:1n-7	3.20^{a}	0.06	3.08^{bc}	0.03	3.07^{b}	0.05	3.03^{c}	0.02
16:2n-4	0.57^{a}	0.00	0.50^{b}	0.01	0.52^{c}	0.00	0.49^{b}	0.01
16:3n-4	1.40^{a}	0.00	1.24^{b}	0.01	1.28^{c}	0.00	1.19^{d}	0.01
17:0	0.71^{a}	0.00	0.68^{b}	0.01	0.70^{a}	0.00	0.65^{c}	0.01
17:1	0.78^{a}	0.00	0.70^{b}	0.00	0.71^{b}	0.00	0.68^{c}	0.01
18:0	4.61^{a}	0.01	4.07^{b}	0.03	4.17^{c}	0.00	3.93^{d}	0.03
18:1n-9	21.53	0.82	23.04	0.73	22.74	0.93	23.00	0.51
18:1n-7	5.38^{a}	0.03	4.72^{b}	0.06	4.79^{b}	0.00	4.49^{c}	0.02
18:2n-6	5.29^{a}	0.02	4.99^{b}	0.03	5.13^{c}	0.01	4.88^{d}	0.04
18:3n-6	0.23^{a}	0.01	0.20^{b}	0.00	0.21^{b}	0.00	0.20^{b}	0.00
18:3n-3	23.43^{a}	0.67	21.21^{b}	0.84	21.71^{ab}	0.82	20.44^{b}	0.77
18:4n-6	3.14^{a}	0.01	2.78^{b}	0.02	2.87^{c}	0.00	2.70^{d}	0.03
18:4n-3	0.16	0.00	0.14	0.00	0.15	0.00	0.14	0.00
20:0	0.14^{a}	0.00	0.17^{ab}	0.03	0.19^{b}	0.00	0.19^{b}	0.00
20:1n-9	0.63^{a}	0.01	0.59^{bc}	0.01	0.62^{ad}	0.01	0.60^{cd}	0.01
20:2n-6	0.21^{a}	0.00	0.13^{b}	0.00	0.19^{c}	0.00	0.18^{c}	0.00
20:4n-6	0.94^{a}	0.01	0.88^{b}	0.00	0.90^{c}	0.00	0.86^{d}	0.00
20:4n-3	0.69^{a}	0.00	0.63^{b}	0.00	0.65^{c}	0.00	0.60^{d}	0.00
20:5n-3	3.72^{a}	0.01	3.97^{b}	0.02	3.93^{b}	0.05	3.76^{a}	0.04
22:0	0.56^{a}	0.00	0.49^{b}	0.00	0.52^{c}	0.00	0.47^{c}	0.00
22:1n-11	0.11^{a}	0.00	-	-	-	-	-	-
22:2n-6	-	-	-	-	0.11^{a}	0.03	0.34^{b}	0.08
22:4n-3	0.38^{a}	0.00	0.19^{b}	0.06	0.31^{ac}	0.00	0.26^{bc}	0.03
22:5n-3	-	-	0.22^{a}	0.03	0.11^{b}	0.00	0.14^{bc}	0.01
22:6n-3	3.91^{a}	0.24	8.04^{b}	0.50	6.65^{c}	0.24	8.49^{b}	0.37
DHA/EPA	1.05^{a}	0.07	2.03^{b}	0.14	1.69^{c}	0.06	2.26^{b}	0.08
EPA/AA	3.95^{a}	0.02	4.48^{b}	0.01	4.36^{c}	0.01	4.38^{c}	0.03
DHA/AA	4.15^{a}	0.29	9.09^{b}	0.59	7.38^{c}	0.27	9.88^{b}	0.41

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. Standard deviation (SD) values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHASCO, DHA-rich single cell oil; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Appendix 6.15. Fatty acid composition (%) of total lipids of *Artemia franciscana* nauplii enriched for 18 hours with DHASCO emulsions at different concentrations.

Fatty acid	Emulsion concentrations (g/million *Artemia*)							
	0.5		1.0		1.5		2.0	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
12:0	0.37^{a}	0.00	0.62^{b}	0.02	0.51^{c}	0.00	0.87^{d}	0.02
14:0	2.03^{a}	0.01	2.84^{b}	0.01	2.42^{c}	0.01	3.55^{d}	0.06
14:1n-5	0.77^{a}	0.01	0.73^{b}	0.00	0.77^{a}	0.00	0.65^{c}	0.01
15:0	0.18^{a}	0.00	0.16^{b}	0.01	0.18^{a}	0.00	0.15^{b}	0.00
16:0	9.70	0.38	10.01	0.24	9.88	0.33	9.66	0.50
16:1n-7	3.07^{a}	0.02	3.06^{a}	0.01	3.09^{a}	0.05	2.90^{b}	0.03
16:2n-4	0.51^{a}	0.00	0.53^{a}	0.06	0.55^{a}	0.07	0.42^{b}	0.00
16:3n-4	1.25^{a}	0.01	1.16^{b}	0.00	1.23^{a}	0.01	1.04^{c}	0.01
17:0	0.66^{a}	0.04	0.66^{a}	0.00	0.65^{a}	0.03	0.55^{b}	0.03
17:1	0.71^{a}	0.01	0.67^{a}	0.00	0.68^{a}	0.03	0.58^{b}	0.03
18:0	4.10^{a}	0.04	3.95^{b}	0.01	4.04^{a}	0.01	3.53^{c}	0.02
18:1n-9	22.60	0.22	23.73	0.50	22.73	0.37	23.44	0.61
18:1n-7	4.85^{a}	0.02	4.61^{b}	0.04	4.77^{ab}	0.04	4.13^{c}	0.17
18:2n-6	4.71^{a}	0.04	4.49^{b}	0.08	4.76^{a}	0.04	4.30^{c}	0.02
18:3n-6	0.20^{a}	0.00	0.17^{b}	0.01	0.19^{a}	0.01	0.16^{b}	0.00
18:3n-3	21.32^{a}	0.49	18.96^{b}	0.29	21.04^{a}	0.38	17.89^{c}	0.42
18:4n-6	2.78^{a}	0.02	2.41^{b}	0.01	2.74^{a}	0.01	2.36^{c}	0.02
18:4n-3	0.15^{a}	0.00	0.14^{ab}	0.00	0.14^{ab}	0.00	0.13^{b}	0.00
20:0	0.13	0.00	0.13	0.00	0.13	0.00	0.12	0.00
20:1n-9	0.58^{a}	0.01	0.56^{b}	0.00	0.56^{b}	0.00	0.50^{c}	0.00
20:2n-6	0.18^{a}	0.00	0.17^{a}	0.00	0.18^{a}	0.00	0.15^{b}	0.01
20:4n-6	0.85^{a}	0.01	0.76^{b}	0.00	0.84^{a}	0.01	0.75^{b}	0.01
20:4n-3	0.65^{a}	0.03	0.55^{b}	0.00	0.65^{a}	0.00	0.55^{b}	0.01
20:5n-3	4.13^{a}	0.04	3.66^{b}	0.07	3.97^{a}	0.08	3.68^{b}	0.06
22:0	0.51^{a}	0.01	0.49^{b}	0.00	0.49^{b}	0.01	0.44^{c}	0.00
22:4n-3	0.23	0.01	0.34	0.09	0.32	0.08	0.27	0.08
22:5n-3	0.14^{ab}	0.01	0.16^{a}	0.02	0.12^{b}	0.01	0.14^{ab}	0.00
22:6n-3	9.05^{a}	0.18	10.41^{b}	0.11	8.73^{c}	0.08	13.79^{d}	0.06
DHA/EPA	2.19^{a}	0.02	2.84^{b}	0.02	2.20^{a}	0.03	3.75^{c}	0.02
EPA/AA	4.87^{ac}	0.01	4.82^{ab}	0.03	4.75^{b}	0.02	4.93^{c}	0.06
DHA/AA	10.67^{a}	0.14	13.69^{b}	0.20	10.46^{a}	0.19	18.50^{c}	0.15

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. Standard deviation (SD) values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHASCO, DHA-rich single cell oil; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.

Appendix 6.16. Fatty acid composition (%) of total lipids of *Artemia franciscana* nauplii enriched for 24 hours with DHASCO emulsions at different concentrations.

Fatty acid	Emulsion concentrations (g/million *Artemia*)							
	0.5		1.0		1.5		2.0	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
12:0	0.28^{a}	0.00	0.32^{b}	0.02	0.41^{c}	0.01	0.56^{d}	0.01
14:0	1.66^{a}	0.02	1.76^{b}	0.01	2.06^{c}	0.00	2.55^{d}	0.01
14:1n-5	0.83^{a}	0.01	0.84^{a}	0.01	0.80^{b}	0.00	0.69^{c}	0.00
15:0	0.23^{a}	0.00	0.28^{b}	0.01	0.19^{c}	0.00	0.17^{d}	0.00
16:0	10.35^{a}	0.15	10.36^{a}	0.21	10.02^{ab}	0.11	9.70^{b}	0.11
16:1n-7	3.19^{a}	0.04	3.37^{b}	0.05	3.12^{ac}	0.00	3.09^{c}	0.03
16:2n-4	0.61^{a}	0.07	0.61^{a}	0.09	0.59^{a}	0.07	0.45^{b}	0.01
16:3n-4	1.36^{a}	0.02	1.33^{ab}	0.00	1.30^{b}	0.00	1.11^{c}	0.02
17:0	0.77^{a}	0.01	0.78^{a}	0.00	0.72^{a}	0.00	0.57^{b}	0.10
17:1	0.77^{a}	0.00	0.76^{a}	0.00	0.74^{a}	0.01	0.65^{b}	0.01
18:0	4.72^{a}	0.06	4.61^{b}	0.03	4.39^{c}	0.01	3.77^{d}	0.04
18:1n-9	22.25^{ab}	0.16	21.65^{a}	0.47	22.55^{b}	0.19	23.82^{c}	0.24
18:1n-7	5.40^{a}	0.19	5.16^{a}	0.11	5.17^{a}	0.13	4.30^{b}	0.07
18:2n-6	5.31^{a}	0.01	4.83^{b}	0.03	5.00^{c}	0.07	4.76^{b}	0.07
18:3n-6	0.22^{a}	0.00	0.20^{a}	0.01	0.21^{a}	0.00	0.17^{b}	0.01
18:3n-3	22.27^{a}	0.30	21.80^{a}	0.32	22.00^{a}	0.26	18.50^{b}	0.18
18:4n-6	2.90^{a}	0.06	2.83^{a}	0.08	2.83^{a}	0.09	2.36^{b}	0.04
18:4n-3	0.18^{ab}	0.01	0.19^{a}	0.01	0.16^{b}	0.01	0.16^{b}	0.00
20:0	0.15	0.00	0.15	0.00	0.15	0.00	0.14	0.00
20:1n-9	0.63^{a}	0.01	0.67^{b}	0.00	0.60^{c}	0.00	0.59^{c}	0.00
20:2n-6	0.21^{a}	0.00	0.20^{a}	0.00	0.20^{a}	0.00	0.17^{b}	0.01
20:4n-6	0.91^{a}	0.03	0.98^{b}	0.00	0.93^{a}	0.00	0.74^{c}	0.01
20:4n-3	0.65^{a}	0.03	0.65^{a}	0.00	0.66^{a}	0.00	0.54^{b}	0.01
20:5n-3	3.95^{a}	0.05	4.13^{b}	0.06	4.18^{b}	0.03	3.97^{a}	0.03
22:0	0.59^{a}	0.01	0.54^{b}	0.00	0.55^{b}	0.01	0.45^{c}	0.00
22:1n-11	-	-	0.13^{a}	0.00	-	-	-	-
22:4n-3	0.38^{a}	0.01	0.41^{b}	0.02	0.31^{c}	0.00	0.28^{d}	0.00
22:5n-3	-	-	0.13^{a}	0.00	0.10^{b}	0.00	0.17^{c}	0.00
22:6n-3	5.41^{a}	0.10	5.45^{a}	0.14	5.97^{b}	0.16	11.94^{c}	0.12
DHA/EPA	1.37^{ab}	0.01	1.32^{a}	0.03	1.43^{b}	0.05	3.01^{c}	0.01
EPA/AA	4.32^{a}	0.10	4.23^{a}	0.01	4.49^{b}	0.02	5.34^{c}	0.03
DHA/AA	5.92^{a}	0.12	5.59^{a}	0.15	6.41^{b}	0.19	16.06^{c}	0.15

Analyses were carried out in triplicates. – Mean values <0.1 or not detected. Standard deviation (SD) values <0.01 are reported as 0.00. Values in each row with different superscripts are different ($p<0.05$) from one another. DHASCO, DHA-rich single cell oil; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid.